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(54) Title: GLYCEROL LINKED PEGYLATED SUGARS AND GLYCOPEPTIDES

(57) Abstract: The present invention provides conjugates between peptides and PEG moieties through glycerol linkers.



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## PATENT APPLICATION

### GLYCEROL LINKED PEGYLATED SUGARS AND GLYCOPEPTIDES

#### CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] The present application claims priority to U.S. Provisional Patent Application No. 60/828,208, filed on October 4, 2006, which is incorporated herein by reference in its entirety for all purposes.

#### SUMMARY OF THE INVENTION

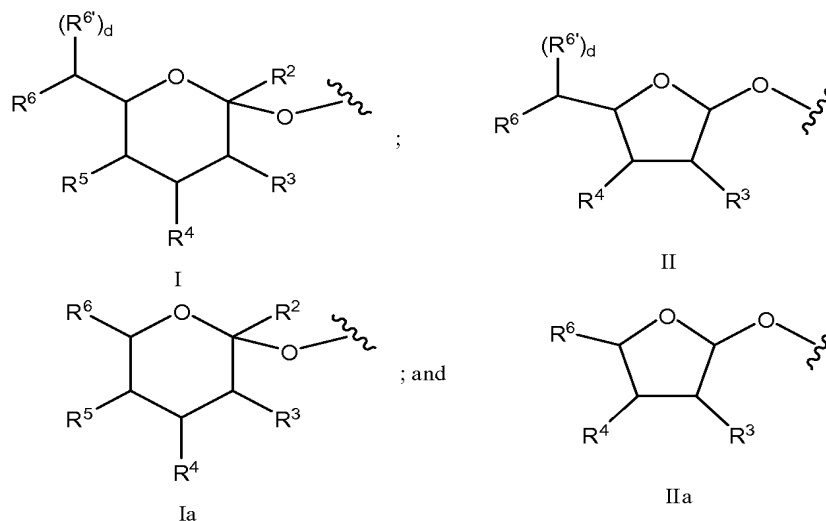
[0002] In an exemplary embodiment, “glycopegylated” molecules of the invention are produced by the enzyme mediated formation of a conjugate between a glycosylated or non-glycosylated peptide and an enzymatically transferable saccharyl moiety that includes a modifying group, such as a polymeric modifying group such as poly(ethylene glycol), within its structure. In an exemplary embodiment, the peptide is a member selected from bone morphogenetic proteins (e.g., BMP-1, BMP-2, BMP-3, BMP-4, BMP-5, BMP-6, BMP-7, BMP-8, BMP-9, BMP-10, BMP-11, BMP-12, BMP-13, BMP-14, BMP-15), neurotrophins (e.g., NT-3, NT-4, NT-5), growth differentiation factors (e.g., GDF-5), glial cell line-derived neurotrophic factor (GDNF), brain derived neurotrophic factor (BDNF), nerve growth factor (NGF), von Willebrand factor (vWF) protease, Factor VII, Factor VIIa, Factor VIII, Factor IX, Factor X, Factor XI, B-domain deleted Factor VIII, vWF-Factor VIII fusion protein having full-length Factor VIII, vWF-Factor VIII fusion protein having B-domain deleted Factor VIII, erythropoietin (EPO), granulocyte colony stimulating factor (G-CSF), Granulocyte-Macrophage Colony Stimulating Factor (GM-CSF), interferon alpha, interferon beta, interferon gamma,  $\alpha_1$ -antitrypsin (ATT, or  $\alpha_1$  protease inhibitor), glucocerebrosidase, Tissue-Type Plasminogen Activator (TPA), Interleukin-2 (IL-2), urokinase, human DNase, insulin, Hepatitis B surface protein (HbsAg), human growth hormone, TNF Receptor-IgG Fc region fusion protein (Enbrel™), anti-HER2 monoclonal antibody (Herceptin™), monoclonal antibody to Protein F of Respiratory Syncytial Virus (Synagis™), monoclonal antibody to TNF- $\alpha$  (Remicade™), monoclonal antibody to glycoprotein IIb/IIIa (Reopro™), monoclonal antibody to CD20 (Rituxan™), anti-thrombin III (AT III), human Chorionic Gonadotropin (hCG), alpha-galactosidase (Fabrazyme™), alpha-iduronidase (Aldurazyme™), follicle stimulating hormone, beta-glucosidase, anti-TNF-alpha monoclonal antibody (MLB 5075),

glucagon-like peptide-1 (GLP-1), glucagon-like peptide-2 (GLP-2), beta-glucosidase, alpha-galactosidase A and fibroblast growth factor. The polymeric modifying group is attached to the saccharyl moiety (i.e., through a single group formed by the reaction of two reactive groups) or through a linker moiety, e.g., substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl, etc.

[0003] Thus, in one aspect, the present invention provides a conjugate between a PEG moiety, e.g., PEG and a peptide that has an *in vivo* activity similar or otherwise analogous to art-recognized therapeutic peptide. In the conjugate of the invention, the PEG moiety is covalently attached to the peptide via an intact glycosyl linking group. Exemplary intact glycosyl linking groups include sialic acid moieties that are derivatized with PEG.

[0004] The polymeric modifying group can be attached at any position of a glycosyl moiety on a peptide. Moreover, the polymeric modifying group can be bound to a glycosyl residue at any position in the amino acid sequence of a wild type or mutant peptide.

[0005] In an exemplary embodiment, the invention provides a peptide that is conjugated through a glycosyl linking group to a polymeric modifying group. Exemplary peptide conjugates include a glycosyl linking group having a formula selected from:

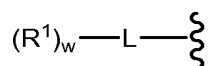


[0006] In Formulae I, Ia, II or IIa, R<sup>2</sup> is H, CH<sub>2</sub>OR<sup>7</sup>, COOR<sup>7</sup>, COO<sup>-</sup> or OR<sup>7</sup>, in which R<sup>7</sup> represents H, substituted or unsubstituted alkyl or substituted or unsubstituted heteroalkyl.

The symbols R<sup>3</sup>, R<sup>4</sup>, R<sup>5</sup>, R<sup>6</sup> and R<sup>6'</sup> independently comprise H, substituted or unsubstituted alkyl, OR<sup>8</sup>, NHC(O)R<sup>9</sup> and a saccharyl moiety. The index d is 0 or 1. R<sup>8</sup> and R<sup>9</sup> are independently selected from H, substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl, sialic acid. At least one of R<sup>3</sup>, R<sup>4</sup>, R<sup>5</sup>, R<sup>6</sup> or R<sup>6'</sup> includes the polymeric

modifying group *e.g.*, PEG. In an exemplary embodiment, R<sup>6</sup> and R<sup>6'</sup>, together with the carbon to which they are attached are components of the side chain of a sialyl moiety. In a further exemplary embodiment, this side chain is functionalized with the polymeric modifying group.

- 5 [0007] In an exemplary embodiment, the polymeric modifying group is bound to the glycosyl linking group, generally through a heteroatom on the glycosyl core (*e.g.*, N, O), through a linker, L, as shown below:



- 10 R<sup>1</sup> is the polymeric modifying group and L is selected from a bond and a linking group. The index w represents an integer selected from 1-6, preferably 1-3 and more preferably 1-2. Exemplary linking groups include substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl moieties and sialic acid. An exemplary component of the linker is an acyl moiety. Another exemplary linking group is an amino acid residue (*e.g.*, cysteine, serine, lysine, and short oligopeptides, *e.g.*, Lys-Lys, Lys-Lys-Lys, Cys-Lys, Ser-Lys, *etc.*).

- 15 [0008] When L is a bond, it is formed by reaction of a reactive functional group on a precursor of R<sup>1</sup> and a reactive functional group of complementary reactivity on a precursor of the glycosyl linking group. When L is a non-zero order linking group, L can be in place on the glycosyl moiety prior to reaction with the R<sup>1</sup> precursor. Alternatively, the precursors of R<sup>1</sup> and L can be incorporated into a preformed cassette that is subsequently attached to the  
20 glycosyl moiety. As set forth herein, the selection and preparation of precursors with appropriate reactive functional groups is within the ability of those skilled in the art. Moreover, coupling of the precursors proceeds by chemistry that is well understood in the art.

- [0009] In an exemplary embodiment L is a linking group that is formed from an amino acid, or small peptide (*e.g.*, 1-4 amino acid residues) providing a modified sugar in which the  
25 polymeric modifying moiety is attached through a substituted alkyl linker. Exemplary linkers include glycine, lysine, serine and cysteine. Amino acid analogs, as defined herein, are also of use as linker components. The amino acid may be modified with an additional component of a linker, *e.g.*, alkyl, heteroalkyl, covalently attached through an acyl linkage, for example, an amide or urethane formed through an amine moiety of the amino acid residue.

- 30 [0010] In an exemplary embodiment, the glycosyl linking group has a structure according to Formulae I or Ia and R<sup>5</sup> includes the polymeric modifying group. In another exemplary



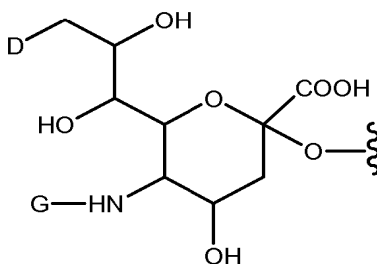
embodiment,  $R^5$  includes both the polymeric modifying group and a linker, L, joining the polymeric modifying group to the glycosyl core. L can be a linear or branched structure. Similarly, the polymeric modifying group can be branched or linear.

[0011] The polymeric modifying group comprises two or more repeating units that can be water-soluble or essentially insoluble in water. Exemplary water-soluble polymers of use in the compounds of the invention include PEG, e.g., m-PEG, PPG, e.g., m-PPG, polysialic acid, polyglutamate, polyaspartate, polylysine, polyethyleneimine, biodegradable polymers (e.g., polylactide, polyglyceride), and functionalized PEG, e.g., terminal-functionalized PEG.

[0012] The glycosyl core of the glycosyl linking groups of use in the peptide conjugates are selected from both natural and unnatural furanoses and pyranoses. The unnatural saccharides optionally include an alkylated or acylated hydroxyl and/or amine moiety, e.g., ethers, esters and amide substituents on the ring. Other unnatural saccharides include an H, hydroxyl, ether, ester or amide substituent at a position on the ring at which such a substituent is not present in the natural saccharide. Alternatively, the carbohydrate is missing a substituent that would be found in the carbohydrate from which its name is derived, e.g., deoxy sugars. Still further exemplary unnatural sugars include both oxidized (e.g., -onic and -uronic acids) and reduced (sugar alcohols) carbohydrates. The sugar moiety can be a mono-, oligo- or poly-saccharide.

[0013] Exemplary natural sugars of use as components of glycosyl linking groups in the present invention include glucose, glucosamine, galactose, galactosamine, fucose, mannose, mannosamine, xylanose, ribose, N-acetyl glucose, N-acetyl glucosamine, N-acetyl galactose, N-acetyl galactosamine, and sialic acid.

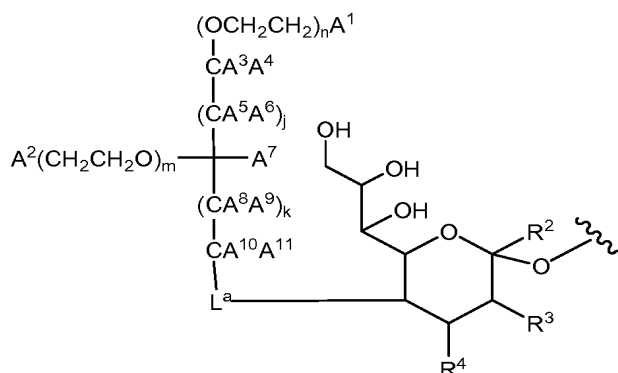
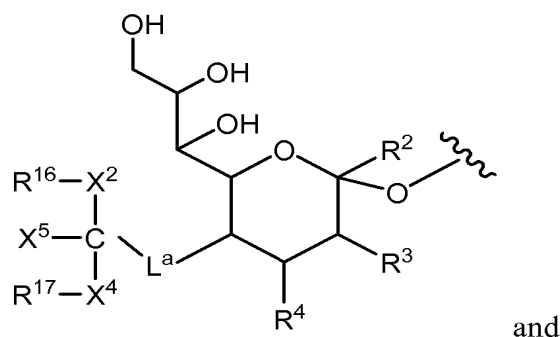
[0014] In one embodiment, the present invention provides a peptide conjugate comprising the moiety:



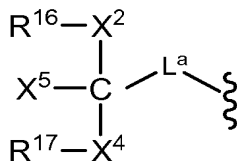
wherein D is a member selected from -OH and  $R^1$ -L-HN-; G is a member selected from H and  $R^1$ -L- and -C(O)(C<sub>1</sub>-C<sub>6</sub>)alkyl;  $R^1$  is a moiety comprising a straight-chain or branched poly(ethylene glycol) residue; and L is a linker, e.g., a bond ("zero order"), substituted or

unsubstituted alkyl and substituted or unsubstituted heteroalkyl. In exemplary embodiments, when D is OH, G is R<sup>1</sup>-L-, and when G is -C(O)(C<sub>1</sub>-C<sub>6</sub>)alkyl, D is R<sup>1</sup>-L-NH-.

[0015] In another aspect, the invention provides a peptide conjugate comprising a glycosyl linking group, wherein the glycosyl linking group is attached to an amino acid residue of said peptide, and wherein said glycosyl linking group comprises a sialyl linking group having a formula which is a member selected from:



wherein



are modifying groups. R<sup>2</sup> is a member selected from H, CH<sub>2</sub>OR<sup>7</sup>, COOR<sup>7</sup>, COO<sup>-</sup> and OR<sup>7</sup>.

R<sup>7</sup> is a member selected from H, substituted or unsubstituted alkyl and substituted or

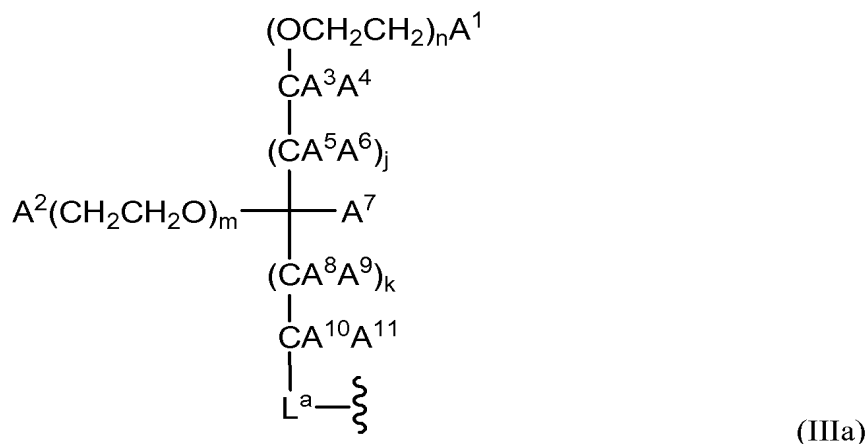
unsubstituted heteroalkyl. R<sup>3</sup> and R<sup>4</sup> are members independently selected from H, substituted or unsubstituted alkyl, OR<sup>8</sup>, and NHC(O)R<sup>9</sup>. R<sup>8</sup> and R<sup>9</sup> are independently selected from H,

substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl and sialyl. L<sup>a</sup> is a linker selected from a bond, substituted or unsubstituted alkyl and substituted or

unsubstituted heteroalkyl. X<sup>5</sup>, R<sup>16</sup> and R<sup>17</sup> are independently selected from non-reactive group and polymeric moieties (e.g. poly(alkylene oxide), e.g., PEG). Non-reactive groups

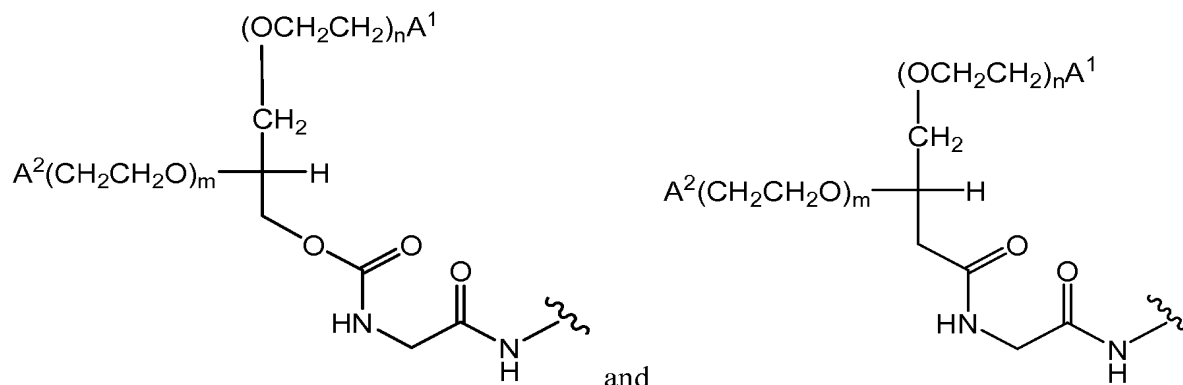
include groups that are considered to be essentially unreactive, neutral and/ or stable at physiological pH, e.g., H, substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl and the like. An exemplary polymeric moiety includes the branched structures set forth in Formula IIIa and its exemplars, below. One of skill in the art will appreciate that the PEG moiety in these formulae can be replaced with other polymers. Exemplary polymers include those of the poly(alkylene oxide) family.  $X^2$  and  $X^4$  are independently selected linkage fragments joining polymeric moieties  $R^{16}$  and  $R^{17}$  to C. The index j is an integer selected from 1 to 15.

[0016] In another exemplary embodiment, the polymeric modifying group has a structure according to the following formula:

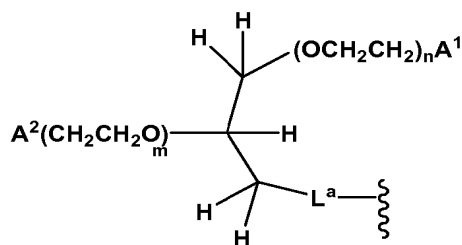


in which the indices m and n are integers independently selected from 0 to 5000.  $\text{A}^1$ ,  $\text{A}^2$ ,  $\text{A}^3$ ,  $\text{A}^4$ ,  $\text{A}^5$ ,  $\text{A}^6$ ,  $\text{A}^7$ ,  $\text{A}^8$ ,  $\text{A}^9$ ,  $\text{A}^{10}$  and  $\text{A}^{11}$  are members independently selected from H, substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl, substituted or unsubstituted cycloalkyl, substituted or unsubstituted heterocycloalkyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl,  $-\text{NA}^{12}\text{A}^{13}$ ,  $-\text{OA}^{12}$  and  $-\text{SiA}^{12}\text{A}^{13}$ .  $\text{A}^{12}$  and  $\text{A}^{13}$  are members independently selected from substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl, substituted or unsubstituted cycloalkyl, substituted or unsubstituted heterocycloalkyl, substituted or unsubstituted aryl, and substituted or unsubstituted heteroaryl.

[0017] In an exemplary embodiment, the polymeric modifying group has a structure including a moiety according to the following formulae:

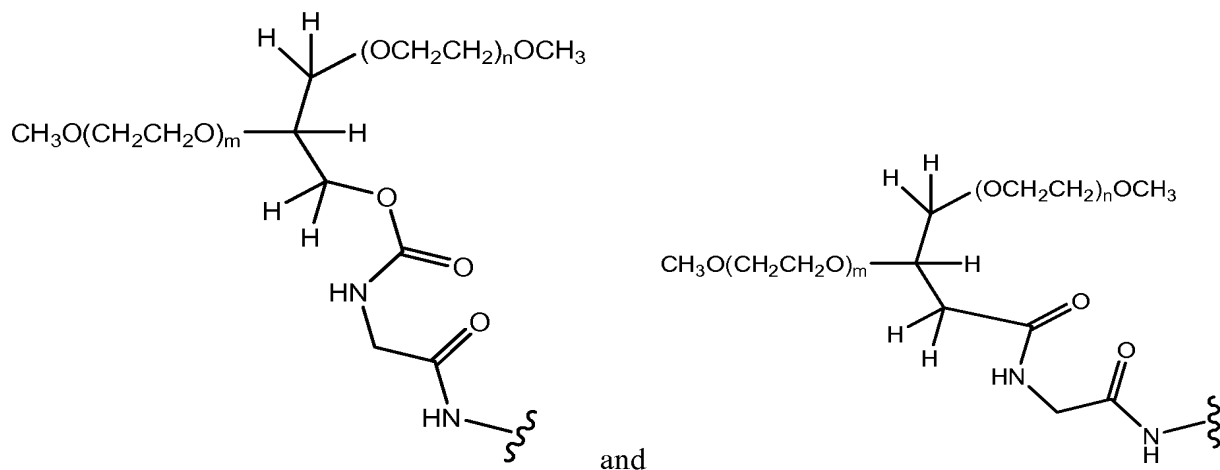


[0018] In another exemplary embodiment according to the formula above, the polymeric modifying group has a structure according to the following formula:

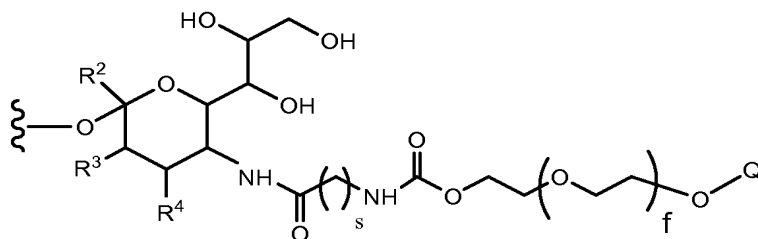


In an exemplary embodiment, m and n are integers independently selected from about 1 to about 5000, preferably from about 100 to about 4000, more preferably from about 200 to about 3000, even more preferably from about 300 to about 2000 and still more preferably from about 400 to about 1000. In an exemplary embodiment, m and n are integers independently selected from about 1 to about 500. In an exemplary embodiment, m and n are integers independently selected from about 1 to about 70, about 70 to about 150, about 150 to about 250, about 250 to about 375 and about 375 to about 500. In an exemplary embodiment, m and n are integers independently selected from about 10 to about 35, about 45 to about 65, about 95 to about 130, about 210 to about 240, about 310 to about 370 and about 420 to about 480. In an exemplary embodiment, m and n are integers selected from about 15 to about 30. In an exemplary embodiment, m and n are integers selected from about 50 to about 65. In an exemplary embodiment, m and n are integers selected from about 100 to about 130. In an exemplary embodiment, m and n are integers selected from about 210 to about 240. In an exemplary embodiment, m and n are integers selected from about 310 to about 370. In an exemplary embodiment, m and n are integers selected from about 430 to about 470. In an exemplary embodiment, A¹ and A² are each members selected from -OH and -OCH₃.

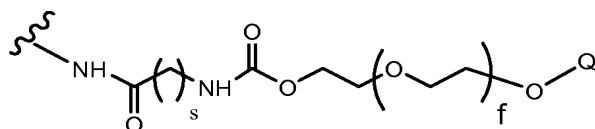
[0019] Exemplary polymeric modifying groups according to this embodiment include the moiety:



[0020] The invention provides a peptide conjugate comprising a glycosyl linking group, wherein the glycosyl linking group is attached to an amino acid residue of the peptide, and wherein the glycosyl linking group comprises a sialyl linking group having the formula:

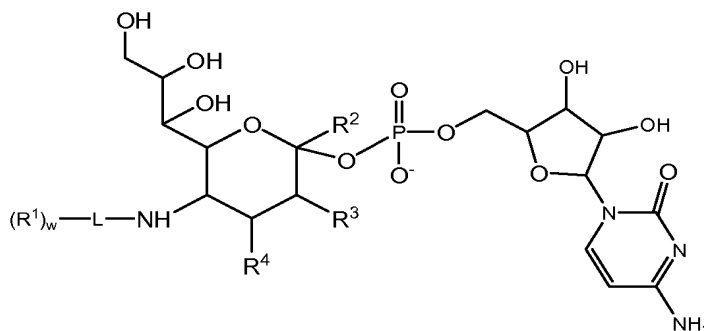


wherein



is a modifying group. The index  $s$  is an integer selected from 1 to 20. The index  $f$  is an integer selected from 1 to 2500.  $Q$  is a member selected from H and substituted or unsubstituted  $C_1$ - $C_6$  alkyl.

[0021] In an exemplary embodiment, the invention provides a modified sugar having the following formula:



wherein  $R^1$  is the polymeric moiety; L is selected from a bond and a linking group;  $R^2$  is a member selected from H,  $\text{CH}_2\text{OR}^7$ ,  $\text{COOR}^7$  and  $\text{OR}^7$ ;  $R^7$  is a member selected from H, substituted or unsubstituted alkyl and substituted or unsubstituted heteroalkyl;  $R^3$  and  $R^4$  are members independently selected from H, substituted or unsubstituted alkyl,  $\text{OR}^8$  and  $\text{NHC(O)R}^9$ ; and  $R^8$  and  $R^9$  are independently selected from H, substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl, sialic acid and polysialic acid. The index w represents an integer selected from 1-6, preferably 1-3 and more preferably 1-2. Exemplary linking groups include substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl moieties and sialic acid. An exemplary component of the linker is an acyl moiety.

[0022] The present invention provides methods of forming conjugates of peptides. The methods include contacting a peptide with a modified sugar donor that bears a modifying group covalently attached to a sugar. The modified sugar moiety is transferred from the donor onto an amino acid or glycosyl residue of the peptide by the action of an enzyme. Representative enzymes include, but are not limited to, glycosyltransferases, e.g., sialyltransferases. The method includes contacting the peptide with: a) a modified sugar donor; and b) an enzyme capable of transferring a modified sugar moiety from the modified sugar donor onto an amino acid or glycosyl residue of the peptide, under conditions appropriate to transfer a modified sugar moiety from the donor to an amino acid or glycosyl residue of the peptide, thereby synthesizing said peptide conjugate.

[0023] In a preferred embodiment, prior to step a), the peptide is contacted with a sialidase, thereby removing at least a portion of the sialic acid on the peptide.

[0024] In another preferred embodiment, the peptide is contacted with a sialidase, a glycosyltransferase and a modified sugar donor. In this embodiment, the peptide is in contact with the sialidase, glycosyltransferase and modified sugar donor essentially simultaneously, no matter the order of addition of the various components. The reaction is carried out under conditions appropriate for the sialidase to remove a sialic acid residue from the peptide; and the glycosyltransferase to transfer a modified sugar moiety from the modified sugar donor to an amino acid or glycosyl residue of the peptide.

[0025] In another preferred embodiment, the desialylation and conjugation are performed in the same vessel, and the desialylated peptide is preferably not purified prior to the conjugation step. In another exemplary embodiment, the method further comprises a 'capping' step involving sialylation of the peptide conjugate. This step is performed in the same reaction vessel that contains the sialidase, sialyltransferase and modified sugar donor without prior purification.

[0026] In another preferred embodiment, the desialylation of the peptide is performed, and the asialo peptide is purified. The purified asialo peptide is then subjected to conjugation reaction conditions. In another exemplary embodiment, the method further comprises a 'capping' step involving sialylation of the peptide conjugate. This step is performed in the same reaction vessel that contains the sialidase, sialyltransferase and modified sugar donor without prior purification.

[0027] In another exemplary embodiment, the capping step, sialylation of the peptide conjugate, is performed in the same reaction vessel that contains the sialidase, sialyltransferase and modified sugar donor without prior purification.

[0028] In an exemplary embodiment, the contacting is for a time less than 20 hours, preferably less than 16 hours, more preferably less than 12 hours, even more preferably less than 8 hours, and still more preferably less than 4 hours.

[0029] In a further aspect, the present invention provides a peptide conjugate reaction mixture. The reaction mixture comprises: a) a sialidase; b) an enzyme which is a member selected from glycosyltransferase, exoglycosidase and endoglycosidase; c) a modified sugar; and d) a peptide.

[0030] In another exemplary embodiment, the ratio of the sialidase to the peptide is selected from 0.1 U/L:2 mg/mL to 10 U/L:1 mg/mL, preferably 0.5 U/L:2 mg/mL, more preferably 1.0 U/L:2 mg/mL, even more preferably 10 U/L:2 mg/mL, still more preferably 0.1 U/L:1 mg/mL, more preferably 0.5 U/L:1 mg/mL, even more preferably 1.0 U/L:1 mg/mL, and still more preferably 10 U/L:1 mg/mL.

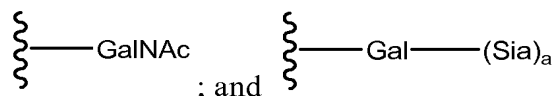
[0031] In an exemplary embodiment, at least 10%, 20%, 30%, 40%, 50%, 60%, 70% or 80% of said peptide conjugate includes at most two PEG moieties. The PEG moieties can be added in a one-pot process, or they can be added after the asialo is purified.

[0032] In another exemplary embodiment, at least 10%, 20%, 30%, 40%, 50%, 60%, 70% or 80% of the peptide conjugate include at most one PEG moiety. The PEG moiety can be added in a one-pot process, or it can be added after the asialo peptide is purified.

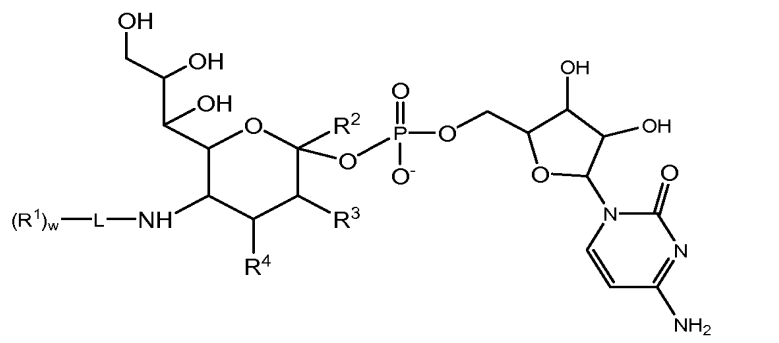
[0033] In a further exemplary embodiment, the method further comprises "capping", or adding sialic acid to the peptide conjugate. In another exemplary embodiment, sialidase is added, followed by a delay of 30 min, 1 hour, 1.5 hours, or 2 hours, followed by the addition of the glycosyltransferase, exoglycosidase, or endoglycosidase.

[0034] In another exemplary embodiment, sialidase is added, followed by a delay of 30 min, 1 hour, 1.5 hours, or 2 hours, followed by the addition of the glycosyltransferase, exoglycosidase, or endoglycosidase. Other objects and advantages of the invention will be apparent to those of skill in the art from the detailed description that follows.

[0035] In another exemplary embodiment, the method includes: (a) contacting a peptide comprising a glycosyl group selected from:



wherein a is an integer from 0 to 10, with a modified sugar having the formula:





and an appropriate transferase which transfers the glycosyl linking group onto a member selected from the GalNAc, Gal and the Sia of said glycosyl group, under conditions appropriate for said transfer. An exemplary modified sugar is CMP-sialic acid modified, through a linker moiety, with a polymer, e.g., a straight chain or branched poly(ethylene glycol) moiety. The radicals in the formula above are substantially the same identity as those found in the identical formula hereinabove.

[0036] The peptide can be acquired from essentially any source, however, in one embodiment, prior to being modified as discussed above, the peptide is expressed in a suitable host. Mammalian (e.g., BHK, CHO), bacteria (e.g., E. coli) and insect cells (e.g., Sf-9) are exemplary expression systems providing a peptide of use in the compositions and methods set forth herein.

[0037] Other objects and advantages of the invention will be apparent to those of skill in the art from the detailed description that follows.

#### DESCRIPTION OF THE DRAWINGS

[0038] FIG. 1 illustrates the preparation of CMP-sialic acid-Glycerol PEG 40 kD.

[0039] FIG. 2 illustrates reaction conditions for the preparation of CMP-sialic acid-Glycerol PEG 40 kD.

[0040] FIG. 3 illustrates the purification process for CMP-sialic acid-Glycerol PEG 40 kD.

[0041] FIG. 4 illustrates the purification process involving Q-Sepharose for CMP-sialic acid-Glycerol PEG 40 kD.

[0042] FIG. 5 is an  $^1\text{H}$  NMR spectra of CMP-sialic acid-Glycerol PEG 40 kD.

[0043] FIG. 6 is a table providing exemplary sialyltransferases of use in forming the glycoconjugates of the invention, e.g., to glycoPEGylate peptides with a modified sialic acid.

[0044] FIG. 7 is a table of the peptides to which one or more glycosyl linking groups can be attached to order to provide the peptide conjugates of the invention.

## DETAILED DESCRIPTION OF THE INVENTION AND THE PREFERRED EMBODIMENTS

### Abbreviations

[0045] PEG, poly(ethyleneglycol); PPG, poly(propyleneglycol); Ara, arabinosyl; Fru, fructosyl; Fuc, fucosyl; Gal, galactosyl; GalNAc, N-acetylgalactosaminyl; Glc, glucosyl; GlcNAc, N-acetylglucosaminyl; Man, mannosyl; ManAc, mannosaminyl acetate; Xyl, xylosyl; NeuAc, sialyl or N-acetylneuraminyl; Sia, sialyl or N-acetylneuraminyl; and derivatives and analogues thereof.

### Definitions

[0046] Unless defined otherwise, all technical and scientific terms used herein generally have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Generally, the nomenclature used herein and the laboratory procedures in cell culture, molecular genetics, organic chemistry and nucleic acid chemistry and hybridization are those well known and commonly employed in the art. Standard techniques are used for nucleic acid and peptide synthesis. The techniques and procedures are generally performed according to conventional methods in the art and various general references (*see generally*, Sambrook *et al.* MOLECULAR CLONING: A LABORATORY MANUAL, 2d ed. (1989) Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., which is incorporated herein by reference), which are provided throughout this document. The nomenclature used herein and the laboratory procedures in analytical chemistry, and organic synthetic described below are those well known and commonly employed in the art. Standard techniques, or modifications thereof, are used for chemical syntheses and chemical analyses.

[0047] All oligosaccharides described herein are described with the name or abbreviation for the non-reducing saccharide (*i.e.*, Gal), followed by the configuration of the glycosidic bond ( $\alpha$  or  $\beta$ ), the ring bond (1 or 2), the ring position of the reducing saccharide involved in the bond (2, 3, 4, 6 or 8), and then the name or abbreviation of the reducing saccharide (*i.e.*, GlcNAc). Each saccharide is preferably a pyranose. For a review of standard glycobiology nomenclature, *see, Essentials of Glycobiology* Varki *et al.* eds. CSHL Press (1999).

[0048] Oligosaccharides are considered to have a reducing end and a non-reducing end, whether or not the saccharide at the reducing end is in fact a reducing sugar. In accordance

with accepted nomenclature, oligosaccharides are depicted herein with the non-reducing end on the left and the reducing end on the right.

[0049] The term “sialic acid” or “sialyl” refers to any member of a family of nine-carbon carboxylated sugars. The most common member of the sialic acid family is N-acetyl-neuraminic acid (2-keto-5-acetamido-3,5-dideoxy-D-glycero-D-galactononulopyranos-1-onic acid (often abbreviated as Neu5Ac, NeuAc, or NANA). A second member of the family is N-glycolyl-neuraminic acid (Neu5Gc or NeuGc), in which the N-acetyl group of NeuAc is hydroxylated. A third sialic acid family member is 2-keto-3-deoxy-nonulosonic acid (KDN) (Nadano *et al.* (1986) *J. Biol. Chem.* **261**: 11550-11557; Kanamori *et al.*, *J. Biol. Chem.* **265**: 21811-21819 (1990)). Also included are 9-substituted sialic acids such as a 9-O-C<sub>1</sub>-C<sub>6</sub> acyl-Neu5Ac like 9-O-lactyl-Neu5Ac or 9-O-acetyl-Neu5Ac, 9-deoxy-9-fluoro-Neu5Ac and 9-azido-9-deoxy-Neu5Ac. For review of the sialic acid family, *see, e.g.*, Varki, *Glycobiology* **2**: 25-40 (1992); *Sialic Acids: Chemistry, Metabolism and Function*, R. Schauer, Ed. (Springer-Verlag, New York (1992)). The synthesis and use of sialic acid compounds in a sialylation procedure is disclosed in international application WO 92/16640, published October 1, 1992.

[0050] “Peptide” refers to a polymer in which the monomers are amino acids and are joined together through amide bonds, alternatively referred to as a polypeptide. Additionally, unnatural amino acids, for example,  $\beta$ -alanine, phenylglycine and homoarginine are also included. Amino acids that are not gene-encoded may also be used in the present invention. Furthermore, amino acids that have been modified to include reactive groups, glycosylation sites, polymers, therapeutic moieties, biomolecules and the like may also be used in the invention. All of the amino acids used in the present invention may be either the D - or L - isomer. The L -isomer is generally preferred. In addition, other peptidomimetics are also useful in the present invention. As used herein, “peptide” refers to both glycosylated and unglycosylated peptides. Also included are peptides that are incompletely glycosylated by a system that expresses the peptide. For a general review, *see*, Spatola, A. F., in *CHEMISTRY AND BIOCHEMISTRY OF AMINO ACIDS, PEPTIDES AND PROTEINS*, B. Weinstein, eds., Marcel Dekker, New York, p. 267 (1983). A listing of some of the peptides of the invention is provided in **FIG. 7**.

[0051] The term “peptide conjugate,” refers to species of the invention in which a peptide is conjugated with a modified sugar as set forth herein.

[0052] The term “amino acid” refers to naturally occurring and synthetic amino acids, as well as amino acid analogs and amino acid mimetics that function in a manner similar to the naturally occurring amino acids. Naturally occurring amino acids are those encoded by the genetic code, as well as those amino acids that are later modified, *e.g.*, hydroxyproline,  $\gamma$ -carboxyglutamate, and O-phosphoserine. Amino acid analogs refers to compounds that have the same basic chemical structure as a naturally occurring amino acid, *i.e.*, an  $\alpha$  carbon that is bound to a hydrogen, a carboxyl group, an amino group, and an R group, *e.g.*, homoserine, norleucine, methionine sulfoxide, methionine methyl sulfonium. Such analogs have modified R groups (*e.g.*, norleucine) or modified peptide backbones, but retain the same basic chemical structure as a naturally occurring amino acid. Amino acid mimetics refers to chemical compounds that have a structure that is different from the general chemical structure of an amino acid, but that function in a manner similar to a naturally occurring amino acid.

[0053] As used herein, the term “modified sugar,” or “modified sugar residue”, refers to a naturally- or non-naturally-occurring carbohydrate that is enzymatically added onto an amino acid or a glycosyl residue of a peptide in a process of the invention. The modified sugar is selected from enzyme substrates including, but not limited to sugar nucleotides (mono-, di-, and tri-phosphates), activated sugars (*e.g.*, glycosyl halides, glycosyl mesylates) and sugars that are neither activated nor nucleotides. The “modified sugar” is covalently functionalized with a “modifying group.” Useful modifying groups include, but are not limited to, PEG moieties, therapeutic moieties, diagnostic moieties, biomolecules and the like. The modifying group is preferably not a naturally occurring, or an unmodified carbohydrate. The locus of functionalization with the modifying group is selected such that it does not prevent the “modified sugar” from being added enzymatically to a peptide.

[0054] As used herein, the term “polymeric moiety” refers to a water-soluble or water-insoluble polymer. The term “water-soluble” refers to moieties that have some detectable degree of solubility in water. Methods to detect and/or quantify water solubility are well known in the art. Exemplary water-soluble polymers include peptides, saccharides, poly(ethers), poly(amines), poly(carboxylic acids) and the like. Peptides can have mixed sequences of be composed of a single amino acid, *e.g.*, poly(lysine). An exemplary polysaccharide is poly(sialic acid). An exemplary poly(ether) is poly(ethylene glycol). Poly(ethylene imine) is an exemplary polyamine, and poly(acrylic) acid is a representative poly(carboxylic acid). Preferred water-soluble polymers are essentially non-fluorescent, or emit such a minimal amount of fluorescence that they are inappropriate for use as a

fluorescent marker in an assay. Polymers that are not naturally occurring sugars may be used. In addition, the use of an otherwise naturally occurring sugar that is modified by covalent attachment of another entity (*e.g.*, poly(ethylene glycol), poly(propylene glycol), poly(aspartate), biomolecule, therapeutic moiety, diagnostic moiety, *etc.*) is also

5 contemplated. The term water-soluble polymer also encompasses species such as saccharides (*e.g.*, dextran, amylose, hyaluronic acid, poly(sialic acid), heparans, heparins, *etc.*); poly(amino acids), *e.g.*, poly(glutamic acid); nucleic acids; synthetic polymers (*e.g.*, poly(acrylic acid), poly(ethers), *e.g.*, poly(ethylene glycol); peptides, proteins, and the like.

Representative water-insoluble polymers include, but are not limited to, polyphosphazines,

10 poly(vinyl alcohols), polyamides, polycarbonates, polyalkylenes, polyacrylamides, polyalkylene glycols, polyalkylene oxides, polyalkylene terephthalates, polyvinyl ethers, polyvinyl esters, polyvinyl halides, polyvinylpyrrolidone, polyglycolides, polysiloxanes, polyurethanes, poly(methyl methacrylate), poly(ethyl methacrylate), poly(butyl methacrylate), poly(isobutyl methacrylate), poly(hexyl methacrylate), poly(isodecyl  
15 methacrylate), poly(lauryl methacrylate), poly(phenyl methacrylate), poly(methyl acrylate), poly(isopropyl acrylate), poly(isobutyl acrylate), poly(octadecyl acrylate) polyethylene, polypropylene, poly(ethylene glycol), poly(ethylene oxide), poly(ethylene terephthalate), poly(vinyl acetate), polyvinyl chloride, polystyrene, polyvinyl pyrrolidone, pluronics and polyvinylphenol and copolymers thereof. In addition, the use of an otherwise naturally  
20 occurring sugar that is modified by covalent attachment of another entity (*e.g.*, poly(ethylene glycol), poly(propylene glycol), poly(aspartate), biomolecule, therapeutic moiety, diagnostic moiety, *etc.*) is also contemplated. Additional examples of water-soluble and water-insoluble polymers are described in the application.

[0055] The polymer backbone of the water-soluble polymer can be poly(ethylene glycol)  
25 (*i.e.* PEG). However, it should be understood that other related polymers are also suitable for use in the practice of this invention and that the use of the term PEG or poly(ethylene glycol) is intended to be inclusive and not exclusive in this respect. The term PEG includes poly(ethylene glycol) in any of its forms, including alkoxy PEG, difunctional PEG, multiarmed PEG, forked PEG, branched PEG, pendent PEG (*i.e.* PEG or related polymers  
30 having one or more functional groups pendent to the polymer backbone), or PEG with degradable linkages therein.

[0056] The polymer can be linear or branched. Branched polymers are generally known in the art. Typically, a branched polymer has a central branch core moiety and a plurality of

linear or branched polymer chains linked to the central branch core. PEG is commonly used in branched forms that can be prepared by addition of ethylene oxide to various polyols, such as glycerol, pentaerythritol and sorbitol. The central branch moiety can also be derived from several amino acids, such as lysine. The branched poly(ethylene glycol) can be represented in general form as R(-PEG-OH)<sub>m</sub> in which R represents the core moiety, such as glycerol or pentaerythritol, and m represents the number of arms. Multi-armed PEG molecules, such as those described in U.S. Pat. No. 5,932,462, which is incorporated by reference herein in its entirety, can also be used as the polymer backbone. In an exemplary embodiment, the branched polymer is itself attached to a branching moiety (e.g., cysteine, serine, lysine, and oligomers of lysine).

**[0057]** Many other polymers are also suitable for the invention. Polymer backbones that are non-peptidic and water-soluble, within about 2 to about 300 loci for attachment, are particularly useful in the invention. Examples of suitable polymers include, but are not limited to, other poly(alkylene glycols), such as poly(propylene glycol) ("PPG"), copolymers of ethylene glycol and propylene glycol and the like, poly(oxyethylated polyol), poly(olefinic alcohol), poly(vinylpyrrolidone), poly(hydroxypropylmethacrylamide), poly( $\alpha$ -hydroxy acid), poly(vinyl alcohol), polyphosphazene, polyoxazoline, poly(N-acryloylmorpholine), such as described in U.S. Pat. No. 5,629,384, which is incorporated by reference herein in its entirety, and copolymers, terpolymers, and mixtures thereof. Although the molecular weight of each chain of the polymer backbone can vary, it is typically in the range of from about 100 Da to about 100,000 Da, often from about 6,000 Da to about 80,000 Da.

**[0058]** The "area under the curve" or "AUC", as used herein in the context of administering a peptide drug to a patient, is defined as total area under the curve that describes the concentration of drug in systemic circulation in the patient as a function of time from zero to infinity.

**[0059]** The term "half-life" or " $t_{1/2}$ ", as used herein in the context of administering a peptide drug to a patient, is defined as the time required for plasma concentration of a drug in a patient to be reduced by one half. There may be more than one half-life associated with the peptide drug depending on multiple clearance mechanisms, redistribution, and other mechanisms well known in the art. Usually, alpha and beta half-lives are defined such that the alpha phase is associated with redistribution, and the beta phase is associated with clearance. However, with protein drugs that are, for the most part, confined to the

bloodstream, there can be at least two clearance half-lives. For some glycosylated peptides, rapid beta phase clearance may be mediated via receptors on macrophages, or endothelial cells that recognize terminal galactose, N-acetylgalactosamine, N-acetylglucosamine, mannose, or fucose. Slower beta phase clearance may occur via renal glomerular filtration for molecules with an effective radius < 2 nm (approximately 68 kD) and/or specific or non-specific uptake and metabolism in tissues. GlycoPEGylation may cap terminal sugars (*e.g.*, galactose or N-acetylgalactosamine) and thereby block rapid alpha phase clearance via receptors that recognize these sugars. It may also confer a larger effective radius and thereby decrease the volume of distribution and tissue uptake, thereby prolonging the late beta phase. Thus, the precise impact of glycoPEGylation on alpha phase and beta phase half-lives may vary depending upon the size, state of glycosylation, and other parameters, as is well known in the art. Further explanation of “half-life” is found in Pharmaceutical Biotechnology (1997, DFA Crommelin and RD Sindelar, eds., Harwood Publishers, Amsterdam, pp 101 – 120).

**[0060]** The term “glycoconjugation,” as used herein, refers to the enzymatically mediated conjugation of a modified sugar species to an amino acid or glycosyl residue of a polypeptide, *e.g.*, a G-CSF peptide of the present invention. A subgenus of “glycoconjugation” is “glyco-PEGylation,” in which the modifying group of the modified sugar is poly(ethylene glycol), and alkyl derivative (*e.g.*, m-PEG) or reactive derivative (*e.g.*, H<sub>2</sub>N-PEG, HOOC-PEG) thereof.

**[0061]** The terms “large-scale” and “industrial-scale” are used interchangeably and refer to a reaction cycle that produces at least about 250 mg, preferably at least about 500 mg, and more preferably at least about 1 gram of glycoconjugate at the completion of a single reaction cycle.

**[0062]** The term, “glycosyl linking group,” as used herein refers to a glycosyl residue to which a modifying group (*e.g.*, PEG moiety, therapeutic moiety, biomolecule) is covalently attached; the glycosyl linking group joins the modifying group to the remainder of the conjugate. In the methods of the invention, the “glycosyl linking group” becomes covalently attached to a glycosylated or unglycosylated peptide, thereby linking the agent to an amino acid and/or glycosyl residue on the peptide. A “glycosyl linking group” is generally derived from a “modified sugar” by the enzymatic attachment of the “modified sugar” to an amino acid and/or glycosyl residue of the peptide. The glycosyl linking group can be a saccharide-derived structure that is degraded during formation of modifying group-modified sugar

cassette (e.g., oxidation→Schiff base formation→reduction), or the glycosyl linking group may be intact. An “intact glycosyl linking group” refers to a linking group that is derived from a glycosyl moiety in which the saccharide monomer that links the modifying group and to the remainder of the conjugate is not degraded, e.g., oxidized, e.g., by sodium metaperiodate. “Intact glycosyl linking groups” of the invention may be derived from a naturally occurring oligosaccharide by addition of glycosyl unit(s) or removal of one or more glycosyl unit from a parent saccharide structure.

[0063] The term, “non-glycosidic modifying group”, as used herein, refers to modifying groups which do not include a naturally occurring sugar linked directly to the glycosyl linking group.

[0064] The term “targeting moiety,” as used herein, refers to species that will selectively localize in a particular tissue or region of the body. The localization is mediated by specific recognition of molecular determinants, molecular size of the targeting agent or conjugate, ionic interactions, hydrophobic interactions and the like. Other mechanisms of targeting an agent to a particular tissue or region are known to those of skill in the art. Exemplary targeting moieties include antibodies, antibody fragments, transferrin, HS-glycoprotein, coagulation factors, serum proteins,  $\beta$ -glycoprotein, G-CSF, GM-CSF, M-CSF, EPO and the like.

[0065] As used herein, “therapeutic moiety” means any agent useful for therapy including, but not limited to, antibiotics, anti-inflammatory agents, anti-tumor drugs, cytotoxins, and radioactive agents. “Therapeutic moiety” includes prodrugs of bioactive agents, constructs in which more than one therapeutic moiety is bound to a carrier, e.g., multivalent agents. Therapeutic moiety also includes proteins and constructs that include proteins. Exemplary proteins include, but are not limited to, Granulocyte Colony Stimulating Factor (GCSF), Granulocyte Macrophage Colony Stimulating Factor (GMCSF), Interferon (e.g., Interferon- $\alpha$ , - $\beta$ , - $\gamma$ ), Interleukin (e.g., Interleukin II), serum proteins (e.g., Factors VII, VIIa, VIII, IX, and X), Human Chorionic Gonadotropin (HCG), Follicle Stimulating Hormone (FSH) and Lutenizing Hormone (LH) and antibody fusion proteins (e.g. Tumor Necrosis Factor Receptor ((TNFR)/Fc domain fusion protein)).

[0066] As used herein, “pharmaceutically acceptable carrier” includes any material, which when combined with the conjugate retains the conjugates’ activity and is non-reactive with the subject's immune systems. Examples include, but are not limited to, any of the



standard pharmaceutical carriers such as a phosphate buffered saline solution, water, emulsions such as oil/water emulsion, and various types of wetting agents. Other carriers may also include sterile solutions, tablets including coated tablets and capsules. Typically such carriers contain excipients such as starch, milk, sugar, certain types of clay, gelatin, stearic acid or salts thereof, magnesium or calcium stearate, talc, vegetable fats or oils, gums, glycols, or other known excipients. Such carriers may also include flavor and color additives or other ingredients. Compositions comprising such carriers are formulated by well known conventional methods.

[0067] As used herein, "administering," means oral administration, administration as a suppository, topical contact, intravenous, intraperitoneal, intramuscular, intralesional, intranasal or subcutaneous administration, or the implantation of a slow-release device *e.g.*, a mini-osmotic pump, to the subject. Administration is by any route including parenteral, and transmucosal (*e.g.*, oral, nasal, vaginal, rectal, or transdermal). Parenteral administration includes, *e.g.*, intravenous, intramuscular, intra-arteriole, intradermal, subcutaneous, intraperitoneal, intraventricular, and intracranial. Moreover, where injection is to treat a tumor, *e.g.*, induce apoptosis, administration may be directly to the tumor and/or into tissues surrounding the tumor. Other modes of delivery include, but are not limited to, the use of liposomal formulations, intravenous infusion, transdermal patches, etc.

[0068] The term "ameliorating" or "ameliorate" refers to any indicia of success in the treatment of a pathology or condition, including any objective or subjective parameter such as abatement, remission or diminishing of symptoms or an improvement in a patient's physical or mental well-being. Amelioration of symptoms can be based on objective or subjective parameters; including the results of a physical examination and/or a psychiatric evaluation.

[0069] The term "therapy" refers to "treating" or "treatment" of a disease or condition including preventing the disease or condition from occurring in an animal that may be predisposed to the disease but does not yet experience or exhibit symptoms of the disease (prophylactic treatment), inhibiting the disease (slowing or arresting its development), providing relief from the symptoms or side-effects of the disease (including palliative treatment), and relieving the disease (causing regression of the disease).

[0070] The term "effective amount" or "an amount effective to" or a "therapeutically effective amount" or any grammatically equivalent term means the amount that, when

administered to an animal for treating a disease, is sufficient to effect treatment for that disease.

[0071] The term “isolated” refers to a material that is substantially or essentially free from components, which are used to produce the material. For peptide conjugates of the invention, the term “isolated” refers to material that is substantially or essentially free from components which normally accompany the material in the mixture used to prepare the peptide conjugate. “Isolated” and “pure” are used interchangeably. Typically, isolated peptide conjugates of the invention have a level of purity preferably expressed as a range. The lower end of the range of purity for the peptide conjugates is about 60%, about 70% or about 80% and the upper end of the range of purity is about 70%, about 80%, about 90% or more than about 90%.

[0072] When the peptide conjugates are more than about 90% pure, their purities are also preferably expressed as a range. The lower end of the range of purity is about 90%, about 92%, about 94%, about 96% or about 98%. The upper end of the range of purity is about 92%, about 94%, about 96%, about 98% or about 100% purity.

[0073] Purity is determined by any art-recognized method of analysis (*e.g.*, band intensity on a silver stained gel, polyacrylamide gel electrophoresis, HPLC, or a similar means).

[0074] “Essentially each member of the population,” as used herein, describes a characteristic of a population of peptide conjugates of the invention in which a selected percentage of the modified sugars added to a peptide are added to multiple, identical acceptor sites on the peptide. “Essentially each member of the population” speaks to the “homogeneity” of the sites on the peptide conjugated to a modified sugar and refers to conjugates of the invention, which are at least about 80%, preferably at least about 90% and more preferably at least about 95% homogenous.

[0075] “Homogeneity,” refers to the structural consistency across a population of acceptor moieties to which the modified sugars are conjugated. Thus, in a peptide conjugate of the invention in which each modified sugar moiety is conjugated to an acceptor site having the same structure as the acceptor site to which every other modified sugar is conjugated, the peptide conjugate is said to be about 100% homogeneous. Homogeneity is typically expressed as a range. The lower end of the range of homogeneity for the peptide conjugates is about 60%, about 70% or about 80% and the upper end of the range of purity is about 70%, about 80%, about 90% or more than about 90%.

[0076] When the peptide conjugates are more than or equal to about 90% homogeneous, their homogeneity is also preferably expressed as a range. The lower end of the range of homogeneity is about 90%, about 92%, about 94%, about 96% or about 98%. The upper end of the range of purity is about 92%, about 94%, about 96%, about 98% or about 100%

5 homogeneity. The purity of the peptide conjugates is typically determined by one or more methods known to those of skill in the art, e.g., liquid chromatography-mass spectrometry (LC-MS), matrix assisted laser desorption mass time of flight spectrometry (MALDITOF), capillary electrophoresis, and the like.

[0077] “Substantially uniform glycoform” or a “substantially uniform glycosylation pattern,” when referring to a glycopeptide species, refers to the percentage of acceptor moieties that are glycosylated by the glycosyltransferase of interest (*e.g.*, fucosyltransferase). For example, in the case of a  $\alpha$ 1,2 fucosyltransferase, a substantially uniform fucosylation pattern exists if substantially all (as defined below) of the Gal $\beta$ 1,4-GlcNAc-R and sialylated analogues thereof are fucosylated in a peptide conjugate of the invention. In the fucosylated  
15 structures set forth herein, the Fuc-GlcNAc linkage is generally  $\alpha$ 1,6 or  $\alpha$ 1,3, with  $\alpha$ 1,6 generally preferred. It will be understood by one of skill in the art, that the starting material may contain glycosylated acceptor moieties (*e.g.*, fucosylated Gal $\beta$ 1,4-GlcNAc-R moieties). Thus, the calculated percent glycosylation will include acceptor moieties that are glycosylated by the methods of the invention, as well as those acceptor moieties already  
20 glycosylated in the starting material.

[0078] The term “substantially” in the above definitions of “substantially uniform” generally means at least about 40%, at least about 70%, at least about 80%, or more preferably at least about 90%, and still more preferably at least about 95% of the acceptor moieties for a particular glycosyltransferase are glycosylated.

25 [0079] Where substituent groups are specified by their conventional chemical formulae, written from left to right, they equally encompass the chemically identical substituents, which would result from writing the structure from right to left, *e.g.*, -CH<sub>2</sub>O- is intended to also recite -OCH<sub>2</sub>-.

[0080] The term “alkyl,” by itself or as part of another substituent means, unless  
30 otherwise stated, a straight or branched chain, or cyclic hydrocarbon radical, or combination thereof, which may be fully saturated, mono- or polyunsaturated and can include di- and multivalent radicals, having the number of carbon atoms designated (*i.e.* C<sub>1</sub>-C<sub>10</sub> means one to

ten carbons). Examples of saturated hydrocarbon radicals include, but are not limited to, groups such as methyl, ethyl, n-propyl, isopropyl, n-butyl, t-butyl, isobutyl, sec-butyl, cyclohexyl, (cyclohexyl)methyl, cyclopropylmethyl, homologs and isomers of, for example, n-pentyl, n-hexyl, n-heptyl, n-octyl, and the like. An unsaturated alkyl group is one having one or more double bonds or triple bonds. Examples of unsaturated alkyl groups include, but are not limited to, vinyl, 2-propenyl, crotyl, 2-isopentenyl, 2-(butadienyl), 2,4-pentadienyl, 3-(1,4-pentadienyl), ethynyl, 1- and 3-propynyl, 3-butylnyl, and the higher homologs and isomers. The term "alkyl," unless otherwise noted, is also meant to include those derivatives of alkyl defined in more detail below, such as "heteroalkyl." Alkyl groups that are limited to hydrocarbon groups are termed "homoalkyl".

**[0081]** The term "alkylene" by itself or as part of another substituent means a divalent radical derived from an alkane, as exemplified, but not limited, by  $-\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2-$ , and further includes those groups described below as "heteroalkylene." Typically, an alkyl (or alkylene) group will have from 1 to 24 carbon atoms, with those groups having 10 or fewer carbon atoms being preferred in the present invention. A "lower alkyl" or "lower alkylene" is a shorter chain alkyl or alkylene group, generally having eight or fewer carbon atoms.

**[0082]** The terms "alkoxy," "alkylamino" and "alkylthio" (or thioalkoxy) are used in their conventional sense, and refer to those alkyl groups attached to the remainder of the molecule via an oxygen atom, an amino group, or a sulfur atom, respectively.

**[0083]** The term "heteroalkyl," by itself or in combination with another term, means, unless otherwise stated, a stable straight or branched chain, or cyclic hydrocarbon radical, or combinations thereof, consisting of the stated number of carbon atoms and at least one heteroatom selected from the group consisting of O, N, Si and S, and wherein the nitrogen and sulfur atoms may optionally be oxidized and the nitrogen heteroatom may optionally be quaternized. The heteroatom(s) O, N and S and Si may be placed at any interior position of the heteroalkyl group or at the position at which the alkyl group is attached to the remainder of the molecule. Examples include, but are not limited to,  $-\text{CH}_2\text{-CH}_2\text{-O-CH}_3$ ,  $-\text{CH}_2\text{-CH}_2\text{-NH-CH}_3$ ,  $-\text{CH}_2\text{-CH}_2\text{-N(CH}_3\text{)-CH}_3$ ,  $-\text{CH}_2\text{-S-CH}_2\text{-CH}_3$ ,  $-\text{CH}_2\text{-CH}_2\text{-S(O)-CH}_3$ ,  $-\text{CH}_2\text{-CH}_2\text{-S(O)}_2\text{-CH}_3$ ,  $-\text{CH=CH-O-CH}_3$ ,  $-\text{Si(CH}_3\text{)}_3$ ,  $-\text{CH}_2\text{-CH=N-OCH}_3$ , and  $-\text{CH=CH-N(CH}_3\text{)-CH}_3$ . Up to two heteroatoms may be consecutive, such as, for example,  $-\text{CH}_2\text{-NH-OCH}_3$  and  $-\text{CH}_2\text{-O-Si(CH}_3\text{)}_3$ . Similarly, the term "heteroalkylene" by itself or as part of another substituent means a divalent radical derived from heteroalkyl, as exemplified, but not limited by,  $-\text{CH}_2-$

CH<sub>2</sub>-S-CH<sub>2</sub>-CH<sub>2</sub>- and -CH<sub>2</sub>-S-CH<sub>2</sub>-CH<sub>2</sub>-NH-CH<sub>2</sub>-. For heteroalkylene groups, heteroatoms can also occupy either or both of the chain termini (*e.g.*, alkyleneoxy, alkylenedioxy, alkyleneamino, alkylenediamino, and the like). Still further, for alkylene and heteroalkylene linking groups, no orientation of the linking group is implied by the direction in which the formula of the linking group is written. For example, the formula -C(O)<sub>2</sub>R'- represents both -C(O)<sub>2</sub>R'- and -R'C(O)<sub>2</sub>-.

**[0084]** The terms "cycloalkyl" and "heterocycloalkyl", by themselves or in combination with other terms, represent, unless otherwise stated, cyclic versions of "alkyl" and "heteroalkyl", respectively. Additionally, for heterocycloalkyl, a heteroatom can occupy the position at which the heterocycle is attached to the remainder of the molecule. Examples of cycloalkyl include, but are not limited to, cyclopentyl, cyclohexyl, 1-cyclohexenyl, 3-cyclohexenyl, cycloheptyl, and the like. Examples of heterocycloalkyl include, but are not limited to, 1-(1,2,5,6-tetrahydropyridyl), 1-piperidinyl, 2-piperidinyl, 3-piperidinyl, 4-morpholinyl, 3-morpholinyl, tetrahydrofuran-2-yl, tetrahydrofuran-3-yl, tetrahydrothien-2-yl, tetrahydrothien-3-yl, 1-piperazinyl, 2-piperazinyl, and the like.

**[0085]** The terms "halo" or "halogen," by themselves or as part of another substituent, mean, unless otherwise stated, a fluorine, chlorine, bromine, or iodine atom. Additionally, terms such as "haloalkyl," are meant to include monohaloalkyl and polyhaloalkyl. For example, the term "halo(C<sub>1</sub>-C<sub>4</sub>)alkyl" is meant to include, but not be limited to, trifluoromethyl, 2,2,2-trifluoroethyl, 4-chlorobutyl, 3-bromopropyl, and the like.

**[0086]** The term "aryl" means, unless otherwise stated, a polyunsaturated, aromatic, substituent that can be a single ring or multiple rings (preferably from 1 to 3 rings), which are fused together or linked covalently. The term "heteroaryl" refers to aryl groups (or rings) that contain from one to four heteroatoms selected from N, O, and S, wherein the nitrogen and sulfur atoms are optionally oxidized, and the nitrogen atom(s) are optionally quaternized. A heteroaryl group can be attached to the remainder of the molecule through a heteroatom. Non-limiting examples of aryl and heteroaryl groups include phenyl, 1-naphthyl, 2-naphthyl, 4-biphenyl, 1-pyrrolyl, 2-pyrrolyl, 3-pyrrolyl, 3-pyrazolyl, 2-imidazolyl, 4-imidazolyl, pyrazinyl, 2-oxazolyl, 4-oxazolyl, 2-phenyl-4-oxazolyl, 5-oxazolyl, 3-isoxazolyl, 4-isoxazolyl, 5-isoxazolyl, 2-thiazolyl, 4-thiazolyl, 5-thiazolyl, 2-furyl, 3-furyl, 2-thienyl, 3-thienyl, 2-pyridyl, 3-pyridyl, 4-pyridyl, 2-pyrimidyl, 4-pyrimidyl, 5-benzothiazolyl, purinyl, 2-benzimidazolyl, 5-indolyl, 1-isoquinolyl, 5-isoquinolyl, 2-quinoxaliny, 5-quinoxaliny, 3-

quinolyl, tetrazolyl, benzo[b]furanyl, benzo[b]thienyl, 2,3-dihydrobenzo[1,4]dioxin-6-yl, benzo[1,3]dioxol-5-yl and 6-quinolyl. Substituents for each of the above noted aryl and heteroaryl ring systems are selected from the group of acceptable substituents described below.

5 [0087] For brevity, the term “aryl” when used in combination with other terms (*e.g.*, aryloxy, arylthioxy, arylalkyl) includes both aryl and heteroaryl rings as defined above. Thus, the term “arylalkyl” is meant to include those radicals in which an aryl group is attached to an alkyl group (*e.g.*, benzyl, phenethyl, pyridylmethyl and the like) including those alkyl groups in which a carbon atom (*e.g.*, a methylene group) has been replaced by, for  
10 example, an oxygen atom (*e.g.*, phenoxymethyl, 2-pyridyloxymethyl, 3-(1-naphthyloxy)propyl, and the like).

[0088] Each of the above terms (*e.g.*, “alkyl,” “heteroalkyl,” “aryl” and “heteroaryl”) is meant to include both substituted and unsubstituted forms of the indicated radical. Preferred substituents for *each* type of radical are provided below.

15 [0089] Substituents for the alkyl and heteroalkyl radicals (including those groups often referred to as alkylene, alkenyl, heteroalkylene, heteroalkenyl, alkynyl, cycloalkyl, heterocycloalkyl, cycloalkenyl, and heterocycloalkenyl) are generically referred to as “alkyl group substituents,” and they can be one or more of a variety of groups selected from, but not limited to: -OR', =O, =NR', =N-OR', -NR'R'', -SR', -halogen, -SiR'R''R''', -OC(O)R', -  
20 C(O)R', -CO<sub>2</sub>R', -CONR'R'', -OC(O)NR'R'', -NR''C(O)R', -NR'-C(O)NR''R''', -NR''C(O)<sub>2</sub>R', -NR-C(NR'R''R''')=NR''', -NR-C(NR'R'')=NR'', -S(O)R', -S(O)<sub>2</sub>R', -S(O)<sub>2</sub>NR'R'', -NRSO<sub>2</sub>R', -CN and -NO<sub>2</sub> in a number ranging from zero to (2m'+1), where m' is the total number of carbon atoms in such radical. R', R'', R''' and R'''' each preferably independently refer to hydrogen, substituted or unsubstituted heteroalkyl, substituted or  
25 unsubstituted aryl, *e.g.*, aryl substituted with 1-3 halogens, substituted or unsubstituted alkyl, alkoxy or thioalkoxy groups, or arylalkyl groups. When a compound of the invention includes more than one R group, for example, each of the R groups is independently selected as are each R', R'', R''' and R'''' groups when more than one of these groups is present. When R' and R'' are attached to the same nitrogen atom, they can be combined with the nitrogen  
30 atom to form a 5-, 6-, or 7-membered ring. For example, -NR'R'' is meant to include, but not be limited to, 1-pyrrolidinyl and 4-morpholinyl. From the above discussion of substituents, one of skill in the art will understand that the term “alkyl” is meant to include groups

including carbon atoms bound to groups other than hydrogen groups, such as haloalkyl (*e.g.*, -CF<sub>3</sub> and -CH<sub>2</sub>CF<sub>3</sub>) and acyl (*e.g.*, -C(O)CH<sub>3</sub>, -C(O)CF<sub>3</sub>, -C(O)CH<sub>2</sub>OCH<sub>3</sub>, and the like).

**[0090]** Similar to the substituents described for the alkyl radical, substituents for the aryl and heteroaryl groups are generically referred to as “aryl group substituents.” The

5 substituents are selected from, for example: halogen, -OR', =O, =NR', =N-OR', -NR'R'', -SR', -halogen, -SiR'R''R''', -OC(O)R', -C(O)R', -CO<sub>2</sub>R', -CONR'R'', -OC(O)NR'R'', -NR''C(O)R', -NR'-C(O)NR''R''', -NR''C(O)<sub>2</sub>R', -NR-C(NR'R''R''')=NR''', -NR-C(NR'R'')=NR''', -S(O)R', -S(O)<sub>2</sub>R', -S(O)<sub>2</sub>NR'R'', -NRSO<sub>2</sub>R', -CN and -NO<sub>2</sub>, -R', -N<sub>3</sub>, -CH(Ph)<sub>2</sub>, fluoro(C<sub>1</sub>-C<sub>4</sub>)alkoxy, and fluoro(C<sub>1</sub>-C<sub>4</sub>)alkyl, in a number ranging from zero to  
10 the total number of open valences on the aromatic ring system; and where R', R'', R''' and R'''' are preferably independently selected from hydrogen, substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl, substituted or unsubstituted aryl and substituted or unsubstituted heteroaryl. When a compound of the invention includes more than one R group, for example, each of the R groups is independently selected as are each R', R'', R'''  
15 and R'''' groups when more than one of these groups is present. In the schemes that follow, the symbol X represents “R” as described above.

**[0091]** Two of the substituents on adjacent atoms of the aryl or heteroaryl ring may optionally be replaced with a substituent of the formula -T-C(O)-(CRR')<sub>u</sub>-U-, wherein T and U are independently -NR-, -O-, -CRR'- or a single bond, and u is an integer of from 0 to 3.

20 Alternatively, two of the substituents on adjacent atoms of the aryl or heteroaryl ring may optionally be replaced with a substituent of the formula -A-(CH<sub>2</sub>)<sub>r</sub>-B-, wherein A and B are independently -CRR'-, -O-, -NR-, -S-, -S(O)-, -S(O)<sub>2</sub>-, -S(O)<sub>2</sub>NR'- or a single bond, and r is an integer of from 1 to 4. One of the single bonds of the new ring so formed may optionally be replaced with a double bond. Alternatively, two of the substituents on adjacent atoms of  
25 the aryl or heteroaryl ring may optionally be replaced with a substituent of the formula - (CRR')<sub>z</sub>-X-(CR''R''')<sub>d</sub>-, where z and d are independently integers of from 0 to 3, and X is -O-, -NR'-, -S-, -S(O)-, -S(O)<sub>2</sub>-, or -S(O)<sub>2</sub>NR'-. The substituents R, R', R'' and R''' are preferably independently selected from hydrogen or substituted or unsubstituted (C<sub>1</sub>-C<sub>6</sub>)alkyl.

**[0092]** As used herein, the term “heteroatom” is meant to include oxygen (O), nitrogen  
30 (N), sulfur (S) and silicon (Si).

**[0093]** As used herein, Factor VII peptide refers to both Factor VII and Factor VIIa peptides. The terms generally refer to variants and mutants of these peptides, including

addition, deletion, substitution and fusion protein mutants. Where both Factor VII and Factor VIIa are used, the use is intended to be illustrative of two species of the genus "Factor VII peptide".

[0094] The invention is meant to include salts of the compounds of the invention which are prepared with relatively nontoxic acids or bases, depending on the particular substituents found on the compounds described herein. When compounds of the present invention contain relatively acidic functionalities, base addition salts can be obtained by contacting the neutral form of such compounds with a sufficient amount of the desired base, either neat or in a suitable inert solvent. Examples of base addition salts include sodium, potassium, lithium, calcium, ammonium, organic amino, or magnesium salt, or a similar salt. When compounds of the present invention contain relatively basic functionalities, acid addition salts can be obtained by contacting the neutral form of such compounds with a sufficient amount of the desired acid, either neat or in a suitable inert solvent. Examples of acid addition salts include those derived from inorganic acids like hydrochloric, hydrobromic, nitric, carbonic, monohydrogencarbonic, phosphoric, monohydrogenphosphoric, dihydrogenphosphoric, sulfuric, monohydrogensulfuric, hydriodic, or phosphorous acids and the like, as well as the salts derived from relatively nontoxic organic acids like acetic, propionic, isobutyric, maleic, malonic, benzoic, succinic, suberic, fumaric, lactic, mandelic, phthalic, benzenesulfonic, p-tolylsulfonic, citric, tartaric, methanesulfonic, and the like. Also included are salts of amino acids such as arginate and the like, and salts of organic acids like glucuronic or galactunoric acids and the like (see, for example, Berge et al., "Pharmaceutical Salts", Journal of Pharmaceutical Science 66: 1-19 (1977)). Certain specific compounds of the present invention contain both basic and acidic functionalities that allow the compounds to be converted into either base or acid addition salts.

[0095] The neutral forms of the compounds are preferably regenerated by contacting the salt with a base or acid and isolating the parent compounds in the conventional manner. The parent form of the compound differs from the various salt forms in certain physical properties, such as solubility in polar solvents.

[0096] "Salt counterion", as used herein, refers to positively charged ions that associate with a compound of the invention when one of its moieties is negatively charged (e.g. COO<sup>-</sup>). Examples of salt counterions include H<sup>+</sup>, H<sub>3</sub>O<sup>+</sup>, ammonium, potassium, calcium, lithium, magnesium and sodium.



[0097] As used herein, the term “CMP-SA-PEG” is a cytidine monophosphate molecule which is conjugated to a sialic acid which comprises a polyethylene glycol moiety. If a length of the polyethylene glycol chain is not specified, then any PEG chain length is possible (e.g. 1kD, 2 kD, 5 kD, 10 kD, 20 kD, 30 kD, 40 kD). An exemplary CMP-SA-PEG is compound 5 in Scheme 1.

## **I. Introduction**

[0098] To improve the effectiveness of recombinant peptides used for therapeutic purposes, the present invention provides conjugates of glycosylated and unglycosylated peptides with a modifying group. The modifying groups can be selected from polymeric modifying groups such as, e.g., PEG (m-PEG), PPG (m-PPG), etc., therapeutic moieties, diagnostic moieties, targeting moieties and the like. Modification of the peptides, e.g., with a water-soluble polymeric modifying group can improve the stability and retention time of the recombinant peptides in a patient's circulation, and/or reduce the antigenicity of recombinant peptides.

[0099] The peptide conjugates of the invention can be formed by the enzymatic attachment of a modified sugar to the glycosylated or unglycosylated peptide. A glycosylation site and/or a modified glycosyl group provides a locus for conjugating a modified sugar bearing a modifying group to the peptide, e.g., by glycoconjugation.

[0100] The methods of the invention also make it possible to assemble peptide conjugates and glycopeptide conjugates that have a substantially homogeneous derivatization pattern. The enzymes used in the invention are generally selective for a particular amino acid residue, combination of amino acid residues, particular glycosyl residues, or combination of glycosyl residues of the peptide. The methods are also practical for large-scale production of peptide conjugates. Thus, the methods of the invention provide a practical means for large-scale preparation of peptide conjugates having preselected uniform derivatization patterns. The methods are particularly well suited for modification of therapeutic peptides, including but not limited to, glycopeptides that are incompletely glycosylated during production in cell culture cells (e.g., mammalian cells, insect cells, plant cells, fungal cells, yeast cells, or prokaryotic cells) or transgenic plants or animals.

[0101] The present invention also provides conjugates of glycosylated and unglycosylated peptides with increased therapeutic half-life due to, for example, reduced

clearance rate, or reduced rate of uptake by the immune or reticuloendothelial system (RES). Moreover, the methods of the invention provide a means for masking antigenic determinants on peptides, thus reducing or eliminating a host immune response against the peptide.

Selective attachment of targeting agents can also be used to target a peptide to a particular tissue or cell surface receptor that is specific for the particular targeting agent.

[0102] Determining optimal conditions for the preparation of peptide conjugates with water-soluble polymers, e.g., involves the optimization of numerous parameters, which are dependent on the identity of the peptide and of the water-soluble polymer. For example, when the polymer is poly(ethylene glycol), e.g., a branched poly(ethylene glycol), a balance is preferably established between the amount of polymer utilized in the reaction and the viscosity of the reaction mixture attributable to the presence of the polymer: if the polymer is too highly concentrated, the reaction mixture becomes viscous, slowing the rate of mass transfer and reaction.

[0103] Furthermore, though it is intuitively apparent to add an excess of enzyme, the present inventors have recognized that, when the enzyme is present in too great of an excess, the excess enzyme becomes a contaminant whose removal requires extra purification steps and material and unnecessarily increases the cost of the final product.

[0104] Moreover, it is generally desired to produce a peptide with a controlled level of modification. In some instances, it is desirable to add one modified sugar preferentially. In other instances, it is desirable to add two modified sugars preferentially. Thus, the reaction conditions are preferably controlled to influence the degree of conjugation of the modifying groups to the peptide.

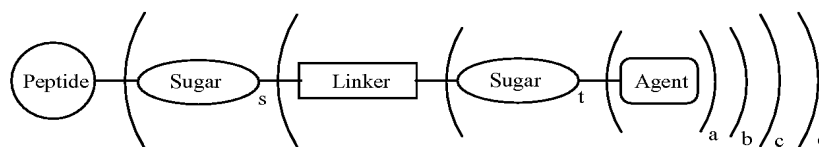
[0105] The present invention provides conditions under which the yield of a peptide, having the desired level of conjugation, is maximized. The conditions in the exemplary embodiments of the inventions also recognize the expense of the various reagents and the materials and time necessary to purify the product: the reaction conditions set forth herein are optimized to provide excellent yields of the desired product, while minimizing waste of costly reagents.

## **II. The Compositions of Matter/Peptide Conjugates**

[0106] In a first aspect, the present invention provides a conjugate between a modified sugar and a peptide. The present invention also provides a conjugate between a modifying

group and a peptide. A peptide conjugate can have one of several forms. In an exemplary embodiment, a peptide conjugate can comprise a peptide and a modifying group linked to an amino acid of the peptide through a glycosyl linking group. In another exemplary embodiment, a peptide conjugate can comprise a peptide and a modifying group linked to a glycosyl residue of the peptide through a glycosyl linking group. In another exemplary embodiment, the peptide conjugate can comprise a peptide and a glycosyl linking group which is bound to both a glycopeptide carbohydrate and directly to an amino acid residue of the peptide backbone. In yet another exemplary embodiment, a peptide conjugate can comprise a peptide and a modifying group linked directly to an amino acid residue of the peptide. In this embodiment, the peptide conjugate may not comprise a glycosyl group. In any of these embodiments, the peptide may or not be glycosylated.

[0107] The conjugates of the invention will typically correspond to the general structure:



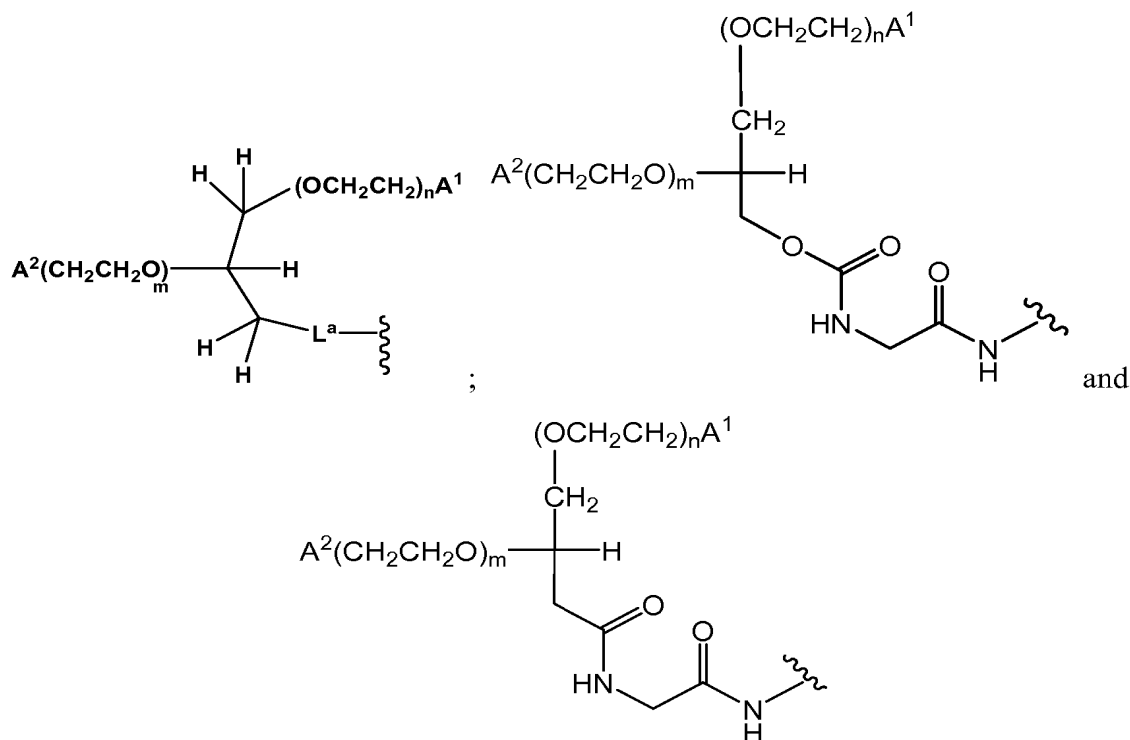
in which the symbols a, b, c, d and s represent a positive, non-zero integer; and t is either 0 or a positive integer. The “agent”, or modifying group, can be a therapeutic agent, a bioactive agent, a detectable label, a polymeric modifying group such as a water-soluble polymer (*e.g.*, PEG, m-PEG, PPG, and m-PPG) or the like. The “agent”, or modifying group, can be a peptide, *e.g.*, enzyme, antibody, antigen, etc. The linker can be any of a wide array of linking groups, *infra*. Alternatively, the linker may be a single bond or a “zero order linker.”

## II. A. Peptide

[0108] The peptide in the peptide conjugate is a member selected from the peptides in FIG. 7. In these cases, the peptide in the peptide conjugate is a member selected from bone morphogenetic proteins (*e.g.*, BMP-1, BMP-2, BMP-3, BMP-4, BMP-5, BMP-6, BMP-7, BMP-8, BMP-9, BMP-10, BMP-11, BMP-12, BMP-13, BMP-14, BMP-15), neurotrophins (*e.g.*, NT-3, NT-4, NT-5), growth differentiation factors (*e.g.*, GDF-5), glial cell line-derived neurotrophic factor (GDNF), brain derived neurotrophic factor (BDNF), nerve growth factor (NGF), von Willebrand factor (vWF) protease, Factor VII, Factor VIIa, Factor VIII, Factor IX, Factor X, Factor XI, B-domain deleted Factor VIII, vWF-Factor VIII fusion protein having full-length Factor VIII, vWF-Factor VIII fusion protein having B-domain deleted Factor VIII, erythropoietin (EPO), granulocyte colony stimulating factor (G-CSF),

Granulocyte-Macrophage Colony Stimulating Factor (GM-CSF), interferon alpha, interferon beta, interferon gamma,  $\alpha_1$ -antitrypsin (ATT, or  $\alpha_1$  protease inhibitor, glucocerebrosidase, Tissue-Type Plasminogen Activator (TPA), Interleukin-2 (IL-2), urokinase, human DNase, insulin, Hepatitis B surface protein (HbsAg), human growth hormone, TNF Receptor-IgG Fc region fusion protein (Enbrel™), anti-HER2 monoclonal antibody (Herceptin™), monoclonal antibody to Protein F of Respiratory Syncytial Virus (Synagis™), monoclonal antibody to TNF- $\alpha$  (Remicade™), monoclonal antibody to glycoprotein IIb/IIIa (Reopro™), monoclonal antibody to CD20 (Rituxan™), anti-thrombin III (AT III), human Chorionic Gonadotropin (hCG), alpha-galactosidase (Fabrazyme™), alpha-iduronidase (Aldurazyme™), follicle stimulating hormone, beta-glucosidase, anti-TNF-alpha monoclonal antibody, glucagon-like peptide-1 (GLP-1), glucagon-like peptide-2 (GLP-2), beta-glucosidase, alpha-galactosidase A and fibroblast growth factor. In certain embodiments, the peptide in the peptide conjugate is Factor VIII. In other embodiments, the peptide in the peptide conjugate is interferon alpha.

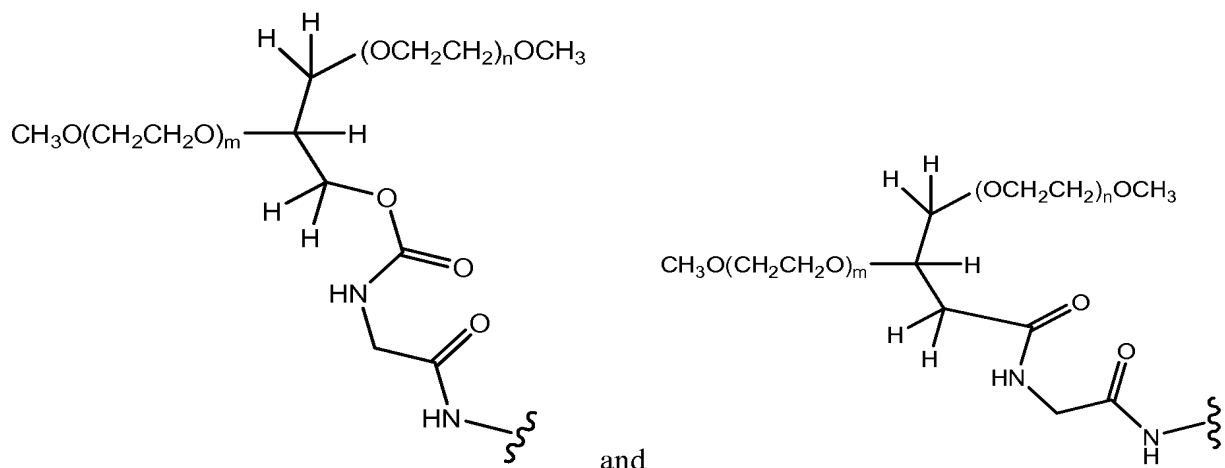
[0109] In an exemplary embodiment, the polymeric modifying group has a structure including a moiety according to the following formulae:



[0110] In an exemplary embodiment, m and n are integers independently selected from about 1 to about 5000, preferably from about 100 to about 4000, more preferably from about 200 to about 3000, even more preferably from about 300 to about 2000 and still more preferably

from about 400 to about 1000 . In an exemplary embodiment, m and n are integers independently selected from about 1 to about 500. In an exemplary embodiment, m and n are integers independently selected from about 1 to about 70, about 70 to about 150, about 150 to about 250, about 250 to about 375 and about 375 to about 500. In an exemplary embodiment, m and n are integers independently selected from about 10 to about 35, about 45 to about 65, about 95 to about 130, about 210 to about 240, about 310 to about 370 and about 420 to about 480. In an exemplary embodiment, m and n are integers selected from about 15 to about 30. In an exemplary embodiment, m and n are integers selected from about 50 to about 65. In an exemplary embodiment, m and n are integers selected from about 100 to about 130. In an exemplary embodiment, m and n are integers selected from about 210 to about 240. In an exemplary embodiment, m and n are integers selected from about 310 to about 370. In an exemplary embodiment, m and n are integers selected from about 430 to about 470. In an exemplary embodiment, A<sup>1</sup> and A<sup>2</sup> are each members selected from -OH and -OCH<sub>3</sub>.

[0111] Exemplary polymeric modifying groups according to this embodiment include the moiety:



[0112] In an exemplary embodiment, in which the modifying group is a branched water-soluble polymer, such as those shown above, it is generally preferred that the concentration of sialidase is about 1.5 to about 2.5 U/L of reaction mixture. More preferably the amount of sialidase is about 2 U/L.

[0113] In another exemplary embodiment, about 5 to about 9 grams of peptide substrate is contacted with the amounts of sialidase set forth above.

[0114] The modified sugar is present in the reaction mixture in an amount from about 1 gram to about 6 grams, preferably from about 3 grams to about 4 grams. It is generally preferred to maintain the concentration of a modified sugar having a branched water-soluble polymer modifying moiety, e.g., the moiety shown above, at less than about 0.5 mM.

5 [0115] In certain embodiments, the modifying group is a branched poly(alkylene oxide), e.g., poly(ethylene glycol), having a molecular weight from about 20 kD to about 60 kD, more preferably, from about 30 kD to about 50 kD, and even more preferably about 40 kD. In other embodiments, the modifying group is a branched poly(alkylene oxide), e.g., poly(ethylene glycol), having a molecular weight of at least about 80 kD, preferably at least  
10 about 100 kD, more preferably at least about 120 kD, at least about 140 kD or at least about 160 kD. In yet another embodiment, the branched poly(alkylene oxide), e.g., poly(ethylene glycol) is at least about 200 kD, such as from at least about 80 kD to at least about 200 kD, including at least about 160 kD and at least about 180 kD. As those of skill will appreciate, the molecular weight of polymers is often polydisperse, thus, the phrase “about” in the  
15 context of molecular weight preferably encompasses a range of values around the stated number. For example, a preferred modifying group having a molecular weight of about 40 kD is one that has a molecular weight from about 35 kD to about 45 kD. Those of skill will appreciate that the reliance on branched PEG structures set forth above is simply for clarity of illustration, the PEG can be replaced by substantially any polymeric moiety, including,  
20 without limitation those species set forth in the definition of “polymeric moiety” found herein.

[0116] Regarding the glycosyltransferase concentration, in a presently preferred embodiment, using the modifying group set forth above, the ratio of glycosyltransferase to peptide is about 40 µg/mL transferase to about 200 µM peptide.

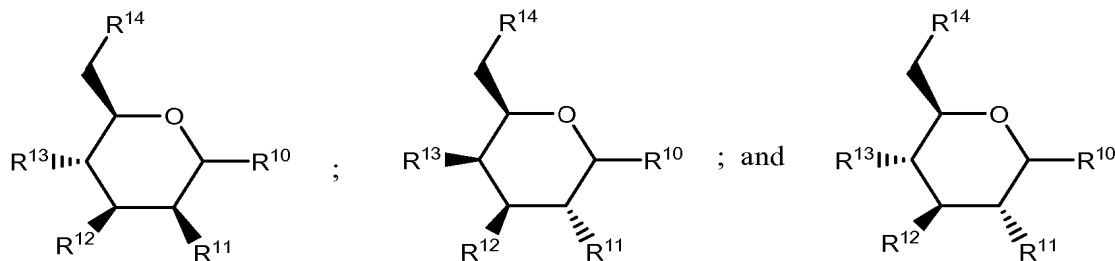
25 **II. B. Modified Sugar**

[0117] In an exemplary embodiment, the peptides of the invention, such as Factor VIII, interferon alpha, and the peptides listed in FIG. 7, are reacted with a modified sugar, thus forming a peptide conjugate. A modified sugar comprises a “sugar donor moiety” as well as a “sugar transfer moiety”. The sugar donor moiety is any portion of the modified sugar that  
30 will be attached to the peptide, either through a glycosyl moiety or amino acid moiety, as a conjugate of the invention. The sugar donor moiety includes those atoms that are chemically altered during their conversion from the modified sugar to the glycosyl linking group of the

peptide conjugate. The sugar transfer moiety is any portion of the modified sugar that will be not be attached to the peptide as a conjugate of the invention. For example, a modified sugar of the invention is the PEGylated sugar nucleotide, PEG-sialic acid CMP. For PEG-sialic acid CMP, the sugar donor moiety, or PEG-sialyl donor moiety, comprises PEG-sialic acid while the sugar transfer moiety, or sialyl transfer moiety, comprises CMP.

[0118] In modified sugars of use in the invention, the saccharyl moiety is preferably a saccharide, a deoxy-saccharide, an amino-saccharide, or an N-acyl saccharide. The term “saccharide” and its equivalents, “saccharyl,” “sugar,” and “glycosyl” refer to monomers, dimers, oligomers and polymers. The sugar moiety is also functionalized with a modifying group. The modifying group is conjugated to the saccharyl moiety, typically, through conjugation with an amine, sulfhydryl or hydroxyl, e.g., primary hydroxyl, moiety on the sugar. In an exemplary embodiment, the modifying group is attached through an amine moiety on the sugar, e.g., through an amide, a urethane or a urea that is formed through the reaction of the amine with a reactive derivative of the modifying group.

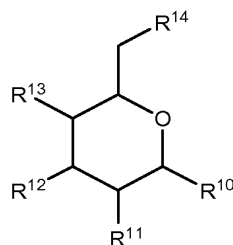
[0119] Any saccharyl moiety can be utilized as the sugar donor moiety of the modified sugar. The saccharyl moiety can be a known sugar, such as mannose, galactose or glucose, or a species having the stereochemistry of a known sugar. The general formulae of these modified sugars are:



Other saccharyl moieties that are useful in forming the compositions of the invention include, but are not limited to fucose and sialic acid, as well as amino sugars such as glucosamine, galactosamine, mannosamine, the 5-amine analogue of sialic acid and the like. The saccharyl moiety can be a structure found in nature or it can be modified to provide a site for conjugating the modifying group. For example, in one embodiment, the modified sugar provides a sialic acid derivative in which the 9-hydroxy moiety is replaced with an amine. The amine is readily derivatized with an activated analogue of a selected modifying group.

[0120] Examples of modified sugars of use in the invention are described in PCT Patent Application No. PCT/US05/002522, which is herein incorporated by reference.

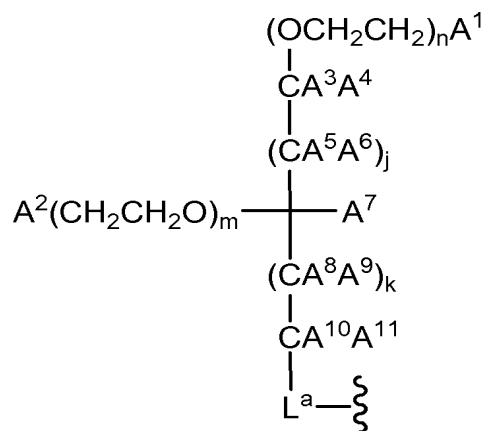
[0121] In a further exemplary embodiment, the invention utilizes modified sugars in which the 6-hydroxyl position is converted to the corresponding amine moiety, which bears a linker-modifying group cassette such as those set forth above. Exemplary glycosyl groups that can be used as the core of these modified sugars include Glu, Gal, GalNAc, Glc, GlcNAc, Fuc, Xyl, Man, and the like. A representative modified sugar according to this



in which R<sup>11</sup>-R<sup>14</sup> are members independently selected from H, OH, C(O)CH<sub>3</sub>, NH, and NH C(O)CH<sub>3</sub>. R<sup>10</sup> is a link to another glycosyl residue (-O-glycosyl) or to an amino acid of the Factor VII/Factor VIIa peptide (-NH-(Factor VII/Factor VIIa)). R<sup>14</sup> is OR<sup>1</sup>, NHR<sup>1</sup> or NH-L-R<sup>1</sup>. R<sup>1</sup> and NH-L-R<sup>1</sup> are as described above.

## II. C. Glycosyl Linking Groups

[0122] In an exemplary embodiment, the invention provides a peptide conjugate formed between a modified sugar of the invention and a peptide. In another exemplary embodiment, when the modifying group on the modified sugar includes the moiety:

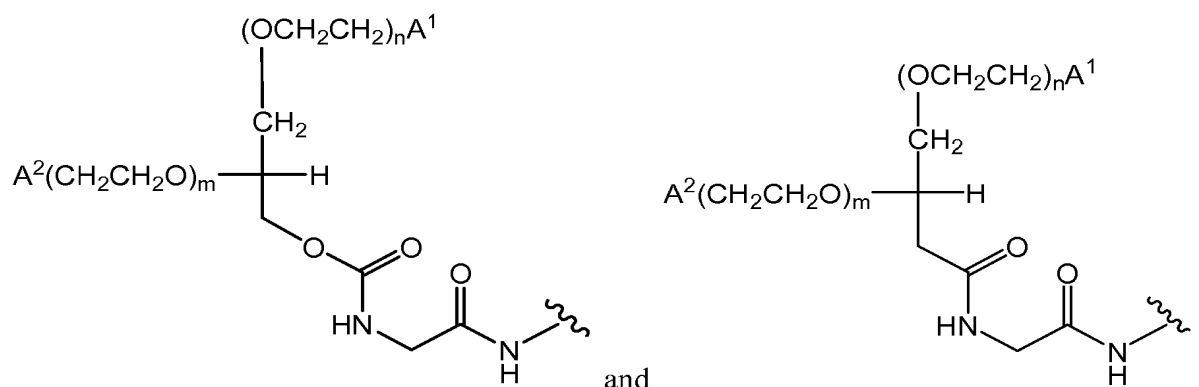


and the peptide in the peptide conjugate is a member selected from the peptides in FIG. 7. In yet another exemplary embodiment, the peptide in the peptide conjugate is a member selected from bone morphogenetic proteins (e.g., BMP-1, BMP-2, BMP-3, BMP-4, BMP-5, BMP-6, BMP-7, BMP-8, BMP-9, BMP-10, BMP-11, BMP-12, BMP-13, BMP-14, BMP-15), neurotrophins (e.g., NT-3, NT-4, NT-5), growth differentiation factors (e.g., GDF-5), glial cell line-derived neurotrophic factor (GDNF), brain derived neurotrophic factor (BDNF),

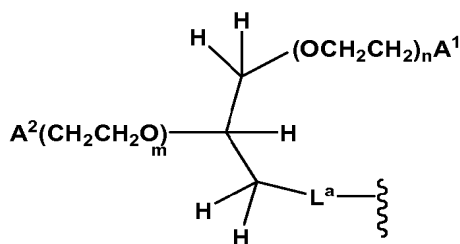


nerve growth factor (NGF), von Willebrand factor (vWF) protease, Factor VII, Factor VIIa, Factor VIII, Factor IX, Factor X, Factor XI, B-domain deleted Factor VIII, vWF-Factor VIII fusion protein having full-length Factor VIII, vWF-Factor VIII fusion protein having B-domain deleted Factor VIII, erythropoietin (EPO), granulocyte colony stimulating factor (G-CSF), Granulocyte-Macrophage Colony Stimulating Factor (GM-CSF), interferon alpha, interferon beta, interferon gamma,  $\alpha_1$ -antitrypsin (ATT, or  $\alpha_1$  protease inhibitor), glucocerebrosidase, Tissue-Type Plasminogen Activator (TPA), Interleukin-2 (IL-2), urokinase, human DNase, insulin, Hepatitis B surface protein (HbsAg), human growth hormone, TNF Receptor-IgG Fc region fusion protein (Enbrel™), anti-HER2 monoclonal antibody (Herceptin™), monoclonal antibody to Protein F of Respiratory Syncytial Virus (Synagis™), monoclonal antibody to TNF- $\alpha$  (Remicade™), monoclonal antibody to glycoprotein IIb/IIIa (Reopro™), monoclonal antibody to CD20 (Rituxan™), anti-thrombin III (AT III), human Chorionic Gonadotropin (hCG), alpha-galactosidase (Fabrazyme™), alpha-iduronidase (Aldurazyme™), follicle stimulating hormone, beta-glucosidase, anti-TNF-alpha monoclonal antibody, glucagon-like peptide-1 (GLP-1), glucagon-like peptide-2 (GLP-2), beta-glucosidase, alpha-galactosidase A and fibroblast growth factor. In certain embodiments the peptide is Factor VIII or interferon alpha. In this embodiment, the sugar donor moiety (such as the saccharyl moiety and the modifying group) of the modified sugar becomes a “glycosyl linking group”. The “glycosyl linking group” can alternatively refer to the glycosyl moiety which is interposed between the peptide and the modifying group.

[0123] In an exemplary embodiment, the polymeric modifying group includes a moiety having a structure according to the following formulae:

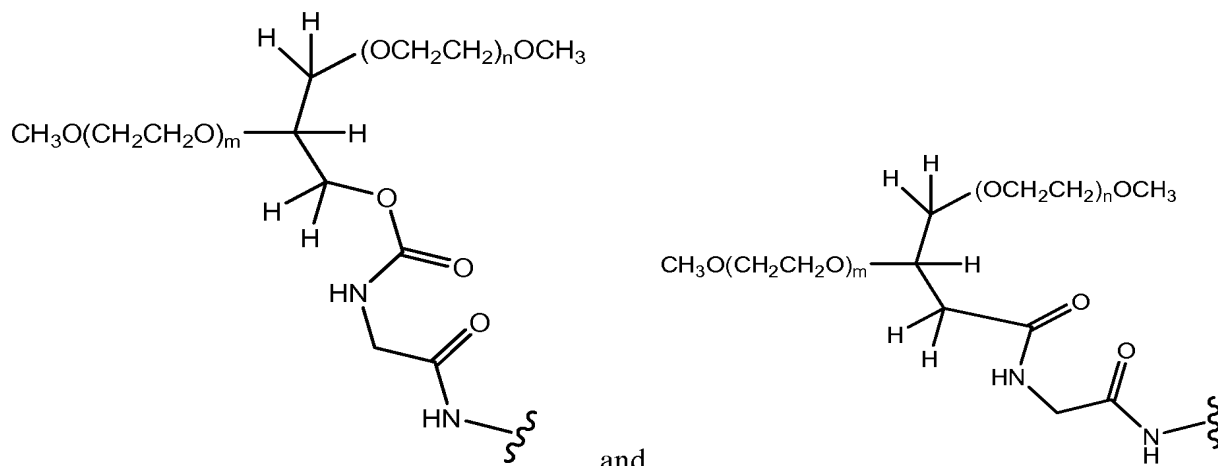


[0124] In an exemplary embodiment, modifying group on the modified sugar includes the moiety:



In an exemplary embodiment, A<sup>1</sup> and A<sup>2</sup> are each members selected from -OH and -OCH<sub>3</sub>.

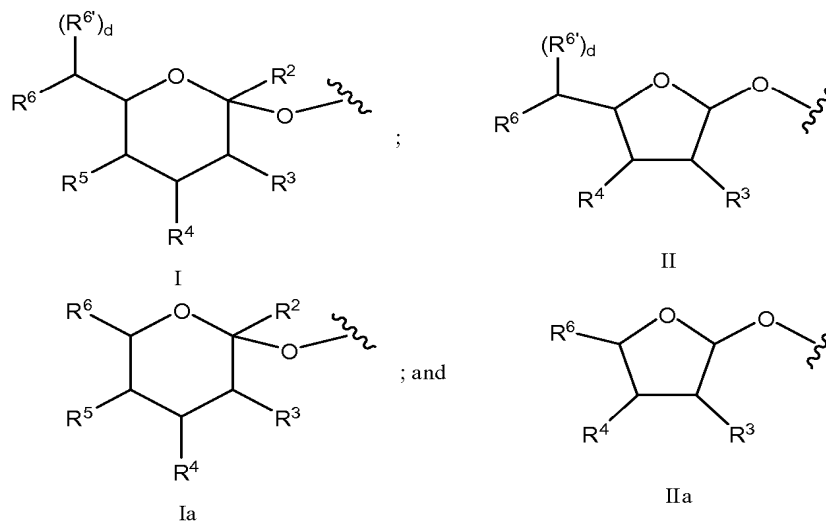
[0125] Exemplary polymeric modifying groups according to this embodiment include the moiety:



[0126] As will be appreciated by those of skill in the art, the PEG moieties in each of the structures shown above can be replaced by any other polymeric moiety, including, without limitation, those species defined herein as “polymeric moieties”.

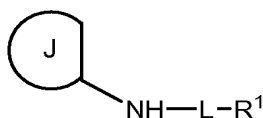
[0127] Due to the versatility of the methods available for adding and/or modifying glycosyl residues on a peptide, the glycosyl linking groups can have substantially any structure. In the discussion that follows, the invention is illustrated by reference to the use of selected derivatives of furanose and pyranose. Those of skill in the art will recognize that the focus of the discussion is for clarity of illustration and that the structures and compositions set forth are generally applicable across the genus of glycosyl linking groups and modified sugars. The glycosyl linking group can comprise virtually any mono- or oligo-saccharide. The glycosyl linking groups can be attached to an amino acid either through the side chain or through the peptide backbone. Alternatively the glycosyl linking groups can be attached to the peptide through a saccharyl moiety. This saccharyl moiety can be a portion of an O-linked or N-linked glycan structure on the peptide.

[0128] In an exemplary embodiment, the invention provides a peptide conjugate comprising an intact glycosyl linking group having a formula that is selected from:



In Formulae I and Ia R<sup>2</sup> is H, CH<sub>2</sub>OR<sup>7</sup>, COOR<sup>7</sup> or OR<sup>7</sup>, in which R<sup>7</sup> represents H, substituted or unsubstituted alkyl or substituted or unsubstituted heteroalkyl. When COOR<sup>7</sup> is a carboxylic acid or carboxylate, both forms are represented by the designation of the single structure COO<sup>-</sup> or COOH. In Formulae I, Ia, II or IIa, the symbols R<sup>3</sup>, R<sup>4</sup>, R<sup>5</sup>, R<sup>6</sup> and R<sup>6'</sup> independently represent H, substituted or unsubstituted alkyl, OR<sup>8</sup>, NHC(O)R<sup>9</sup>. The index d is 0 or 1. R<sup>8</sup> and R<sup>9</sup> are independently selected from H, substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl, sialic acid or polysialic acid. At least one of R<sup>3</sup>, R<sup>4</sup>, R<sup>5</sup>, R<sup>6</sup> or R<sup>6'</sup> includes a modifying group. This modifying group can be a polymeric modifying moiety *e.g.*, PEG, linked through a bond or a linking group. In an exemplary embodiment, R<sup>6</sup> and R<sup>6'</sup>, together with the carbon to which they are attached are components of the pyruvyl side chain of sialic acid. In a further exemplary embodiment, the pyruvyl side chain is functionalized with the polymeric modifying group. In another exemplary embodiment, R<sup>6</sup> and R<sup>6'</sup>, together with the carbon to which they are attached are components of the side chain of sialic acid and the polymeric modifying group is a component of R<sup>5</sup>.

[0129] In an exemplary embodiment, the invention utilizes a glycosyl linking group that has the formula:



in which J is a glycosyl moiety, L is a bond or a linker and R<sup>1</sup> is a modifying group, *e.g.*, a polymeric modifying group. Exemplary bonds are those that are formed between an NH<sub>2</sub>

moiety on the glycosyl moiety and a group of complementary reactivity on the modifying group. For example, when  $R^1$  includes a carboxylic acid moiety, this moiety may be activated and coupled with the  $NH_2$  moiety on the glycosyl residue affording a bond having the structure  $NHC(O)R^1$ . J is preferably a glycosyl moiety that is “intact”, not having been degraded by exposure to conditions that cleave the pyranose or furanose structure, e.g. oxidative conditions, e.g., sodium periodate.

[0130] Exemplary linkers include alkyl and heteroalkyl moieties. The linkers include linking groups, for example acyl-based linking groups, e.g.,  $-C(O)NH-$ ,  $-OC(O)NH-$ , and the like. The linking groups are bonds formed between components of the species of the invention, e.g., between the glycosyl moiety and the linker (L), or between the linker and the modifying group ( $R^1$ ). Other exemplary linking groups are ethers, thioethers and amines. For example, in one embodiment, the linker is an amino acid residue, such as a glycine residue. The carboxylic acid moiety of the glycine is converted to the corresponding amide by reaction with an amine on the glycosyl residue, and the amine of the glycine is converted to the corresponding amide or urethane by reaction with an activated carboxylic acid or carbonate of the modifying group.

[0131] An exemplary species of  $NH-L-R^1$  has the formula:  
 $-NH\{C(O)(CH_2)_aNH\}_s\{C(O)(CH_2)_b(OCH_2CH_2)_cO(CH_2)_dNH\}_tR^1$ , in which the indices s and t are independently 0 or 1. The indices a, b and d are independently integers from 0 to 20, and c is an integer from 1 to 2500. Other similar linkers are based on species in which an  $-NH$  moiety is replaced by another group, for example,  $-S$ ,  $-O$  or  $-CH_2$ . As those of skill will appreciate one or more of the bracketed moieties corresponding to indices s and t can be replaced with a substituted or unsubstituted alkyl or heteroalkyl moiety.

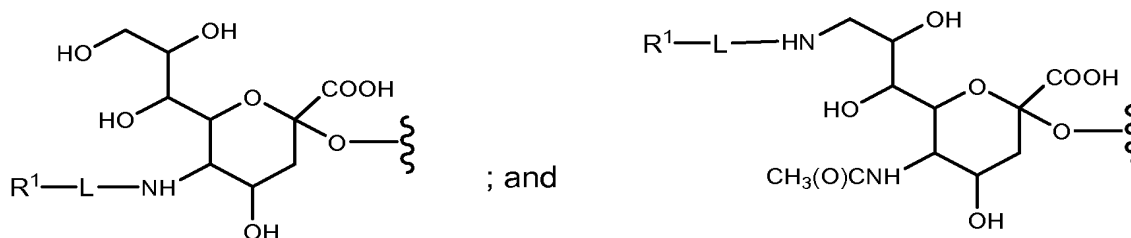
[0132] More particularly, the invention utilizes compounds in which  $NH-L-R^1$  is:

$NHC(O)(CH_2)_aNHC(O)(CH_2)_b(OCH_2CH_2)_cO(CH_2)_dNHR^1$ ,  
 $NHC(O)(CH_2)_b(OCH_2CH_2)_cO(CH_2)_dNHR^1$ ,  $NHC(O)O(CH_2)_b(OCH_2CH_2)_cO(CH_2)_dNHR^1$ ,  
 $NH(CH_2)_aNHC(O)(CH_2)_b(OCH_2CH_2)_cO(CH_2)_dNHR^1$ ,  $NHC(O)(CH_2)_aNHR^1$ ,  
 $NH(CH_2)_aNHR^1$ , and  $NHR^1$ . In these formulae, the indices a, b and d are independently selected from the integers from 0 to 20, preferably from 1 to 5. The index c is an integer from 1 to about 2500.

[0133] In an exemplary embodiment, c is selected such that the PEG moiety is approximately 1 kD, 5 kD, 10, kD, 15 kD, 20 kD, 25 kD, 30 kD, 35 kD, 40 kD or 45 kD.

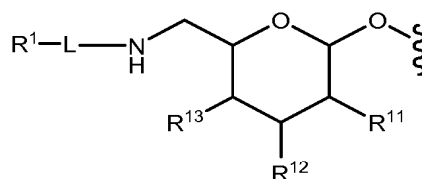
[0134] For the purposes of convenience, the glycosyl linking groups in the remainder of this section will be based on a sialyl moiety. However, one of skill in the art will recognize that another glycosyl moiety, such as mannosyl, galactosyl, glucosyl, or fucosyl, could be used in place of the sialyl moiety.

- 5 [0135] In an exemplary embodiment, the glycosyl linking group is an intact glycosyl linking group, in which the glycosyl moiety or moieties forming the linking group are not degraded by chemical (e.g., sodium metaperiodate) or enzymatic (e.g., oxidase) processes. Selected conjugates of the invention include a modifying group that is attached to the amine moiety of an amino-saccharide, e.g., mannosamine, glucosamine, galactosamine, sialic acid  
 10 etc. Exemplary modifying group-intact glycosyl linking group cassettes according to this motif are based on a sialic acid structure, such as those having the formulae:



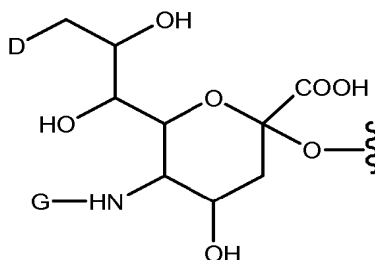
[0136] In the formulae above, R<sup>1</sup> and L are as described above. Further detail about the structure of exemplary R<sup>1</sup> groups is provided below.

- 15 [0137] In still a further exemplary embodiment, the conjugate is formed between a peptide and a modified sugar in which the modifying group is attached through a linker at the 6-carbon position of the modified sugar. Thus, illustrative glycosyl linking groups according to this embodiment have the formula:



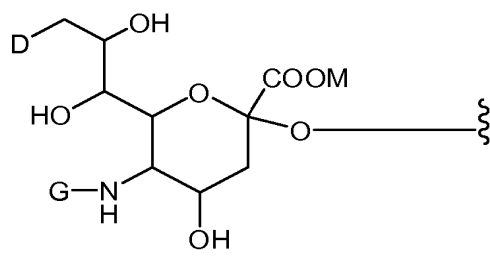
- 20 in which the radicals are as discussed above. Glycosyl linking groups include, without limitation, glucose, glucosamine, N-acetyl-glucosamine, galactose, galactosamine, N-acetyl-galactosamine, mannose, mannosamine, N-acetyl-mannosamine, and the like.

[0138] In one embodiment, the present invention provides a peptide conjugate comprising the following glycosyl linking group:



wherein D is a member selected from -OH and  $R^1$ -L-HN-; G is a member selected from H and  $R^1$ -L- and -C(O)(C<sub>1</sub>-C<sub>6</sub>)alkyl;  $R^1$  is a moiety comprising a straight-chain or branched poly(ethylene glycol) residue; and L is a linker, e.g., a bond ("zero order"), substituted or unsubstituted alkyl and substituted or unsubstituted heteroalkyl. In exemplary embodiments, when D is OH, G is  $R^1$ -L-, and when G is -C(O)(C<sub>1</sub>-C<sub>6</sub>)alkyl, D is  $R^1$ -L-NH-.

**[0139]** In one embodiment, the present invention provides a peptide conjugate comprising the following glycosyl linking group:

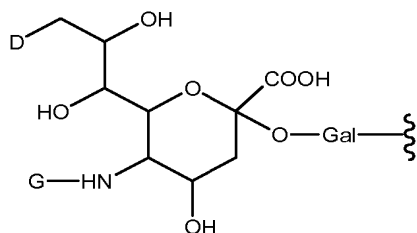


;

D is a member selected from -OH and  $R^1$ -L-HN-; G is a member selected from  $R^1$ -L- and -C(O)(C<sub>1</sub>-C<sub>6</sub>)alkyl- $R^1$ ;  $R^1$  is a moiety comprising a member selected from a straight-chain poly(ethylene glycol) residue and branched poly(ethylene glycol) residue; and M is a member selected from H, a salt counterion and a single negative charge; L is a linker which is a member selected from a bond, substituted or unsubstituted alkyl and substituted or unsubstituted heteroalkyl. In an exemplary embodiment, when D is OH, G is  $R^1$ -L-. In another exemplary embodiment, when G is -C(O)(C<sub>1</sub>-C<sub>6</sub>)alkyl, D is  $R^1$ -L-NH-.

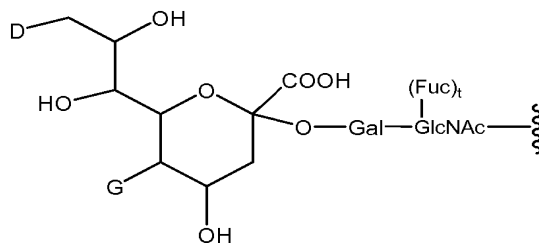
**[0140]** In any the compounds of the invention, a COOH group can alternatively be COOM, wherein M is a member selected from H, a negative charge, and a salt counterion.

**[0141]** The invention provides a peptide conjugate that includes a glycosyl linking group having the formula:



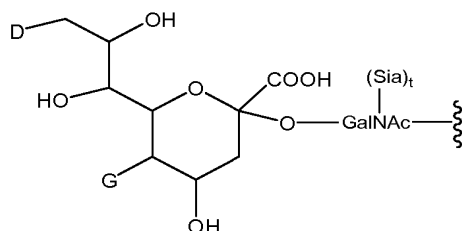
wherein D and G are as described above.

[0142] In other embodiments, the glycosyl linking group has the formula:



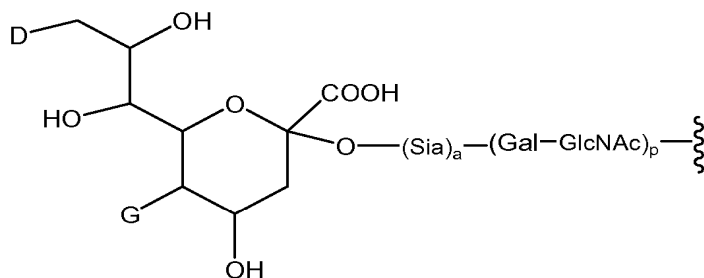
5 wherein D and G are as described above and the index t is 0 or 1.

[0143] In a still further exemplary embodiment, the glycosyl linking group has the formula:



wherein D and G are as described above and the index t is 0 or 1.

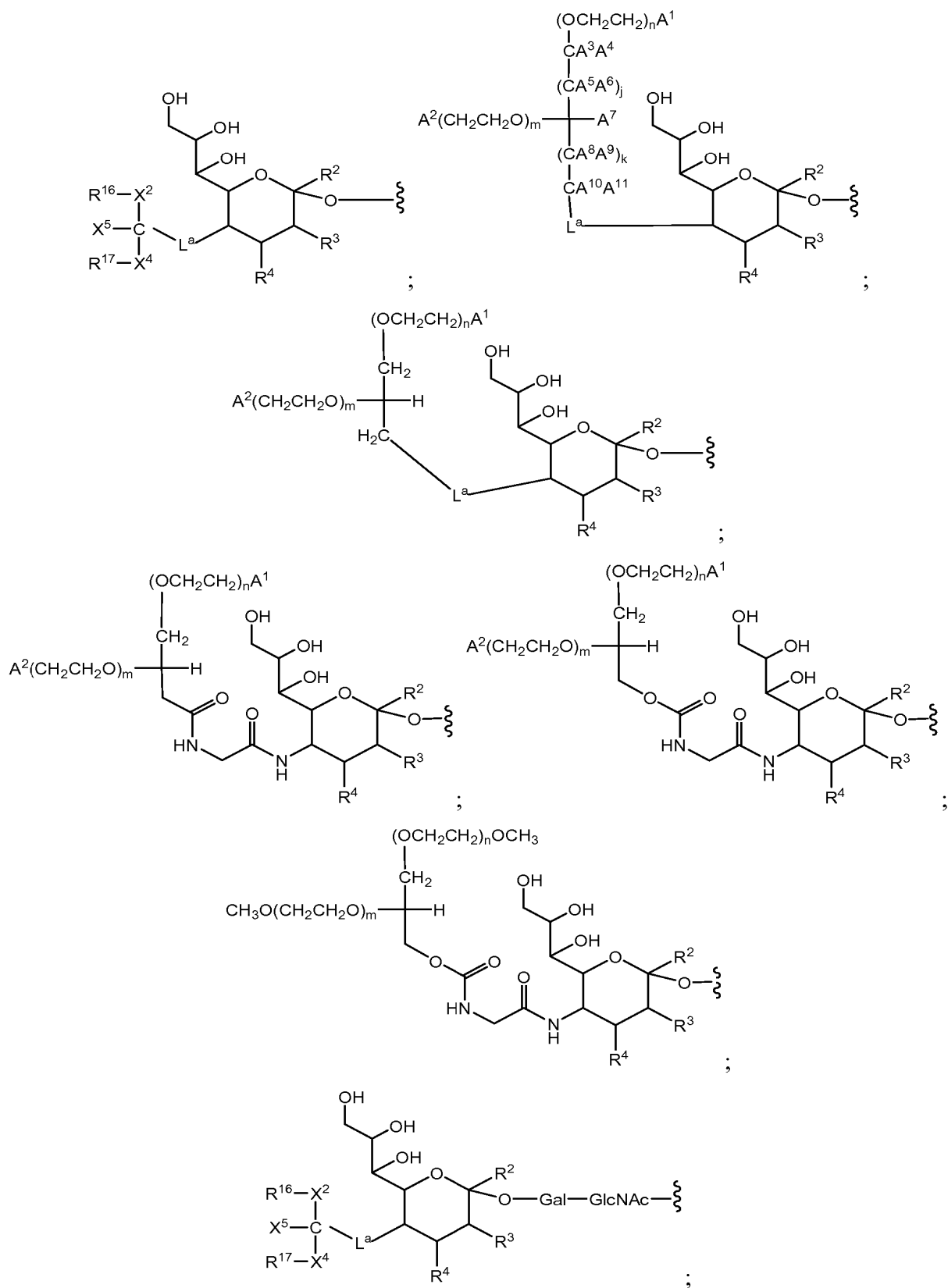
10 [0144] In yet another embodiment, the glycosyl linking group has the formula:



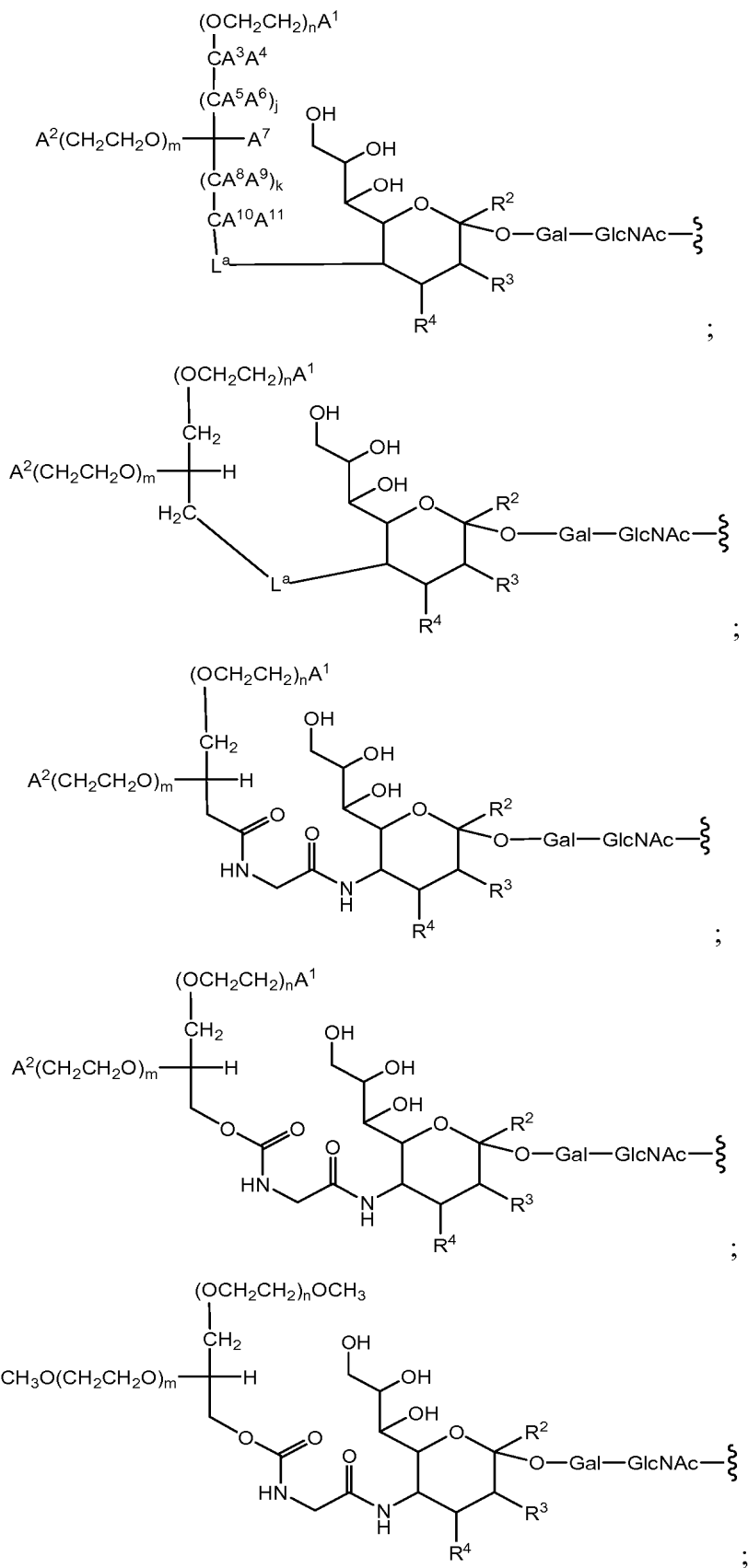
wherein D and G are as described above and the index p represents an integer from 1 to 10; and a is either 0 or 1.

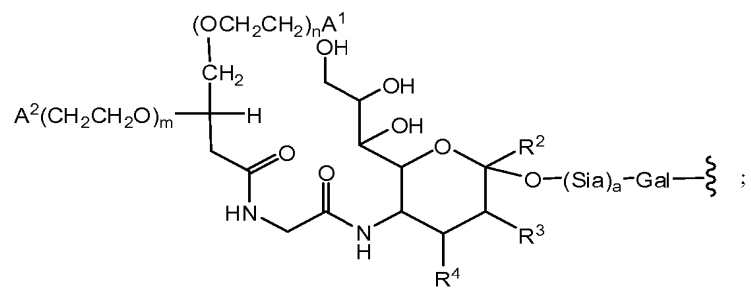
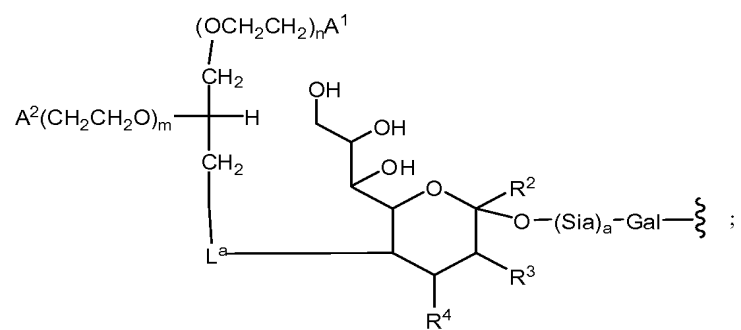
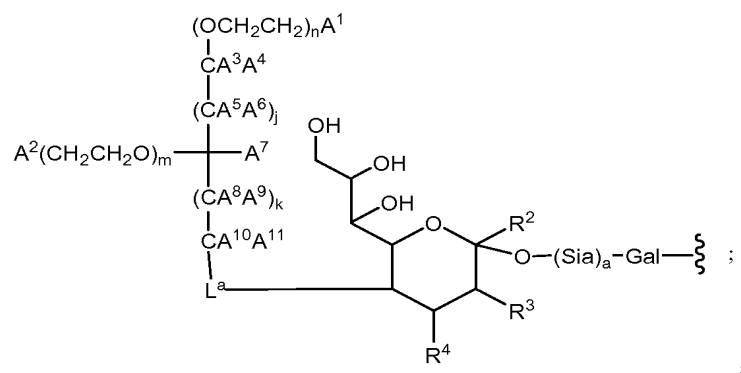
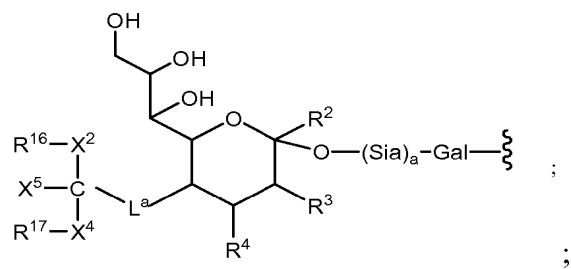
[0145] In another exemplary embodiment, the peptide conjugate comprises a glycosyl moiety selected from the formulae:

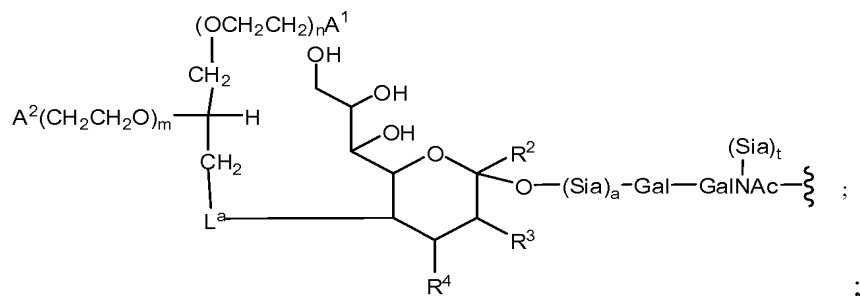
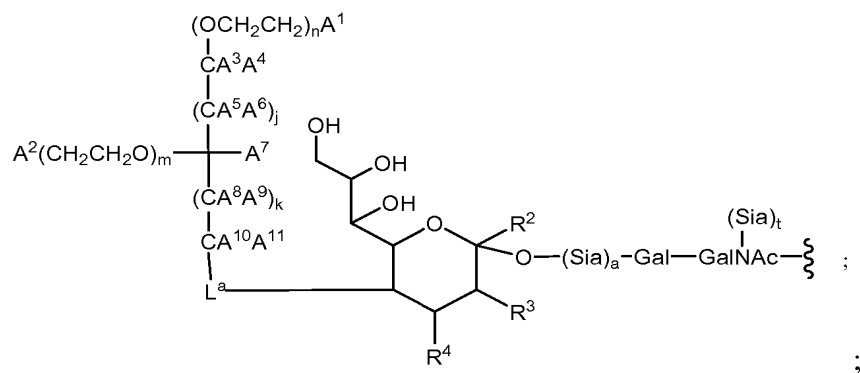
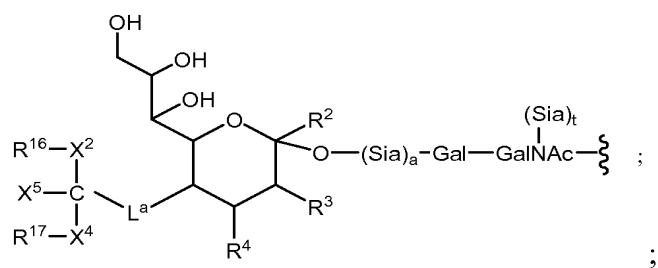
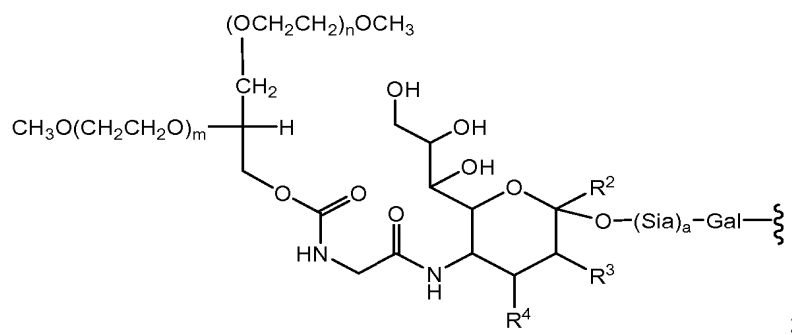
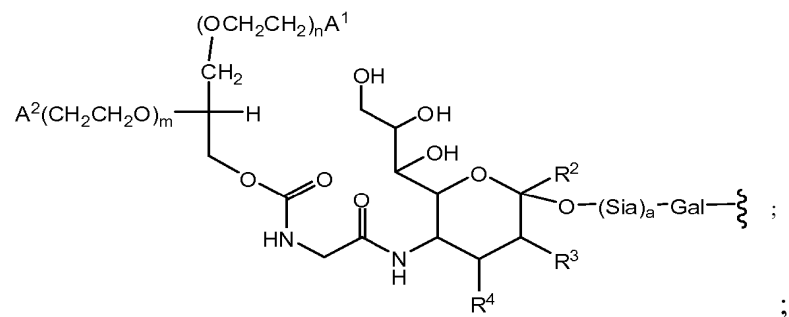
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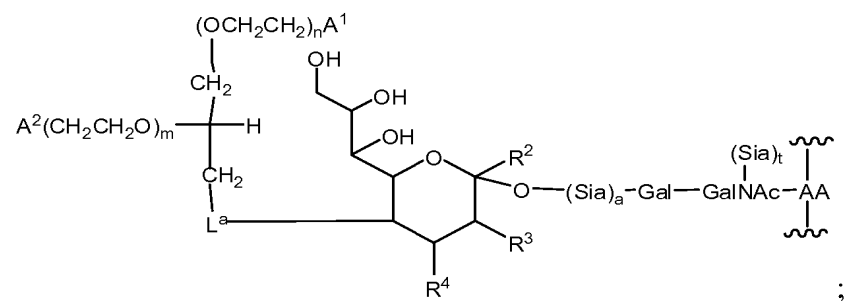
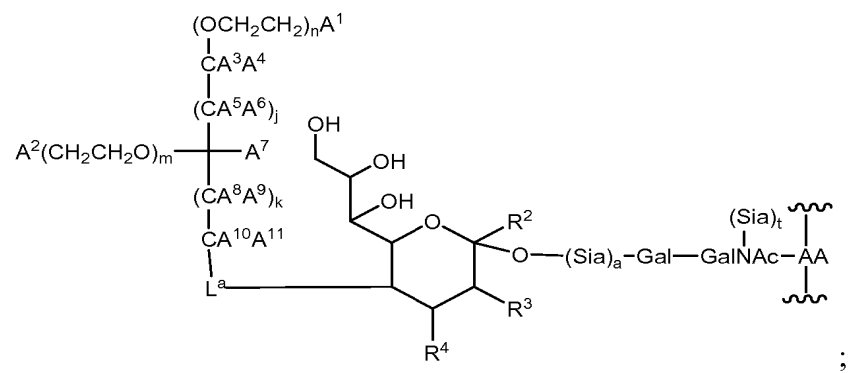
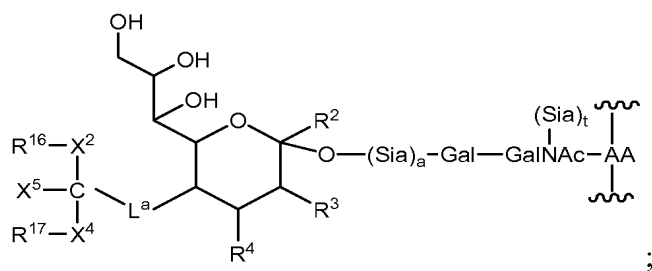
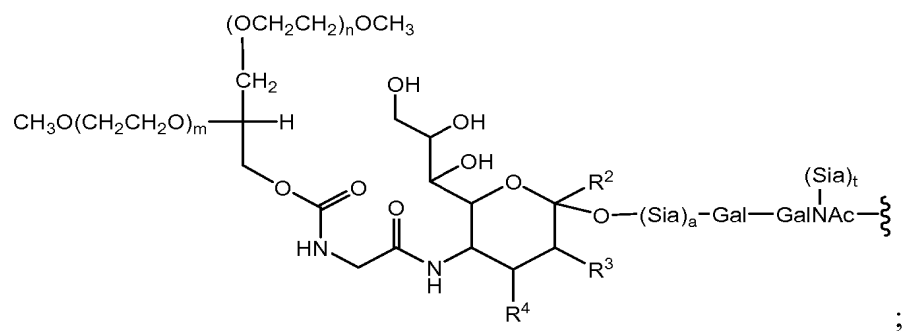
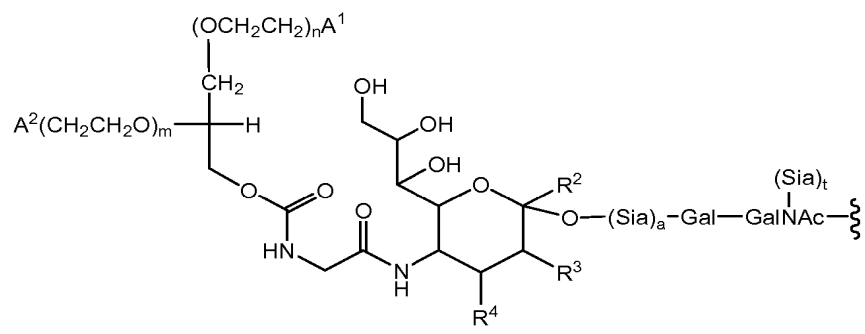


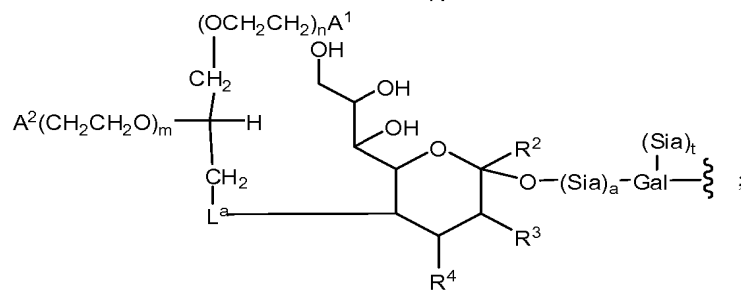
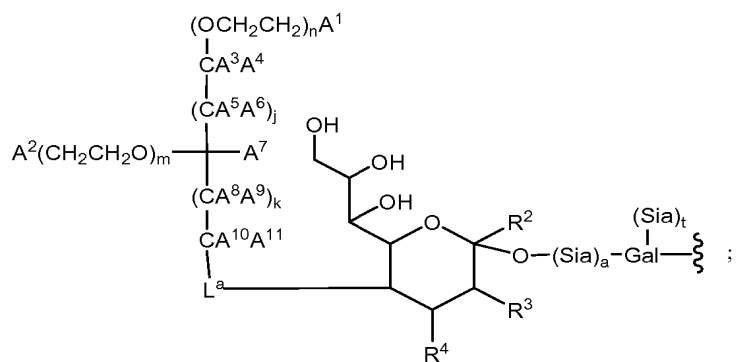
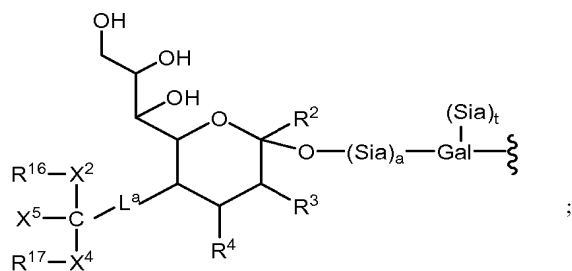
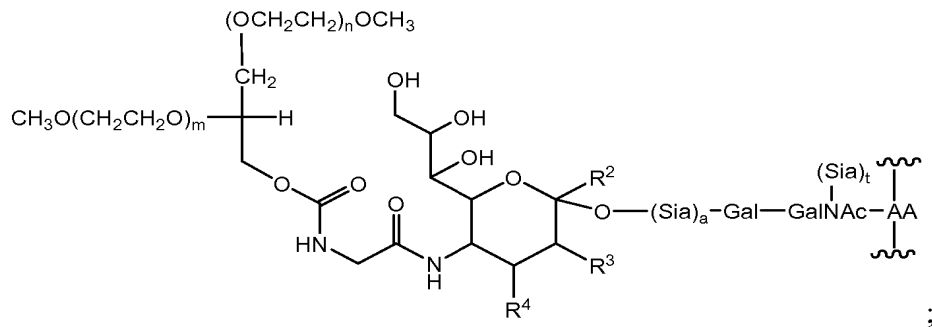
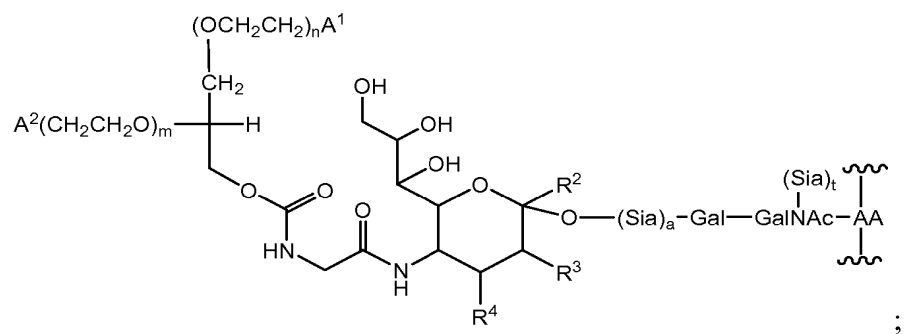


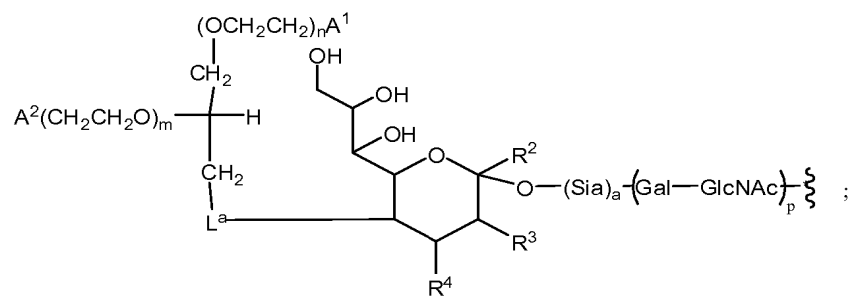
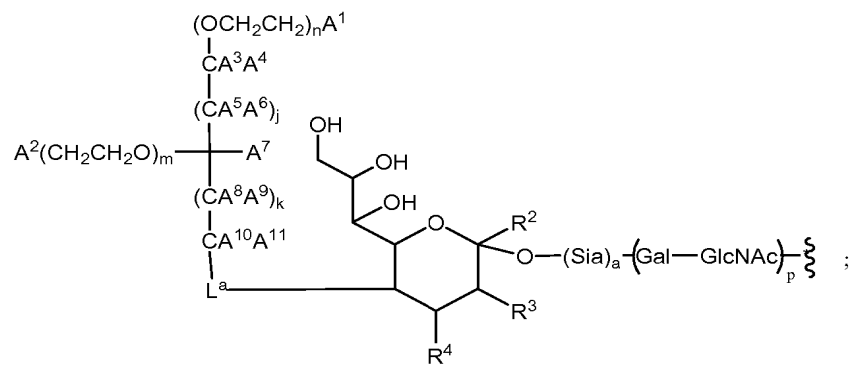
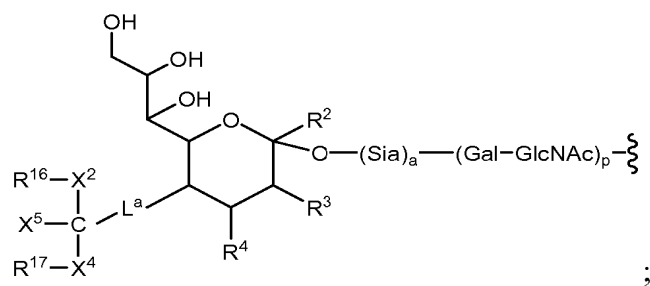
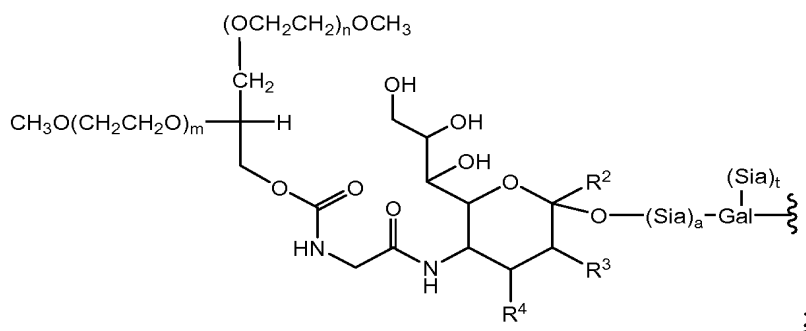
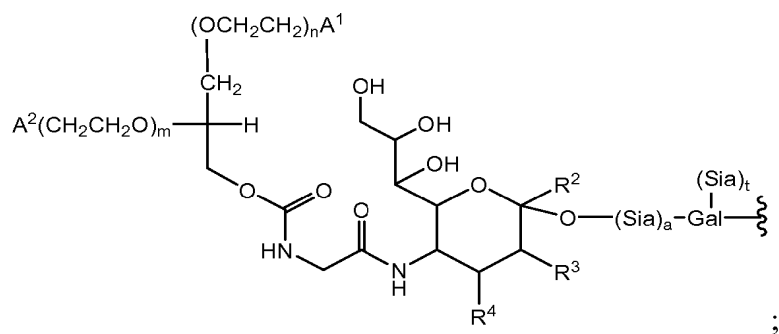


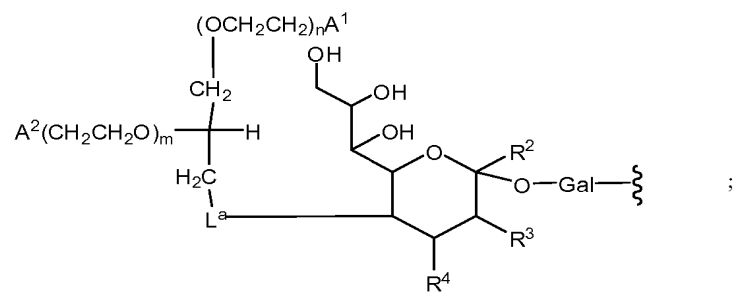
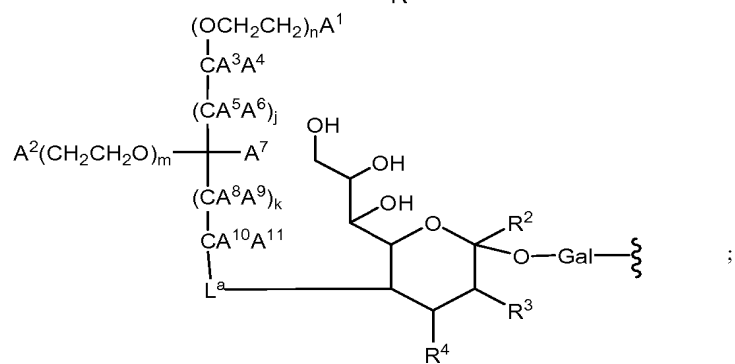
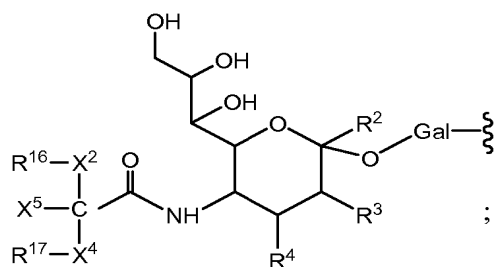
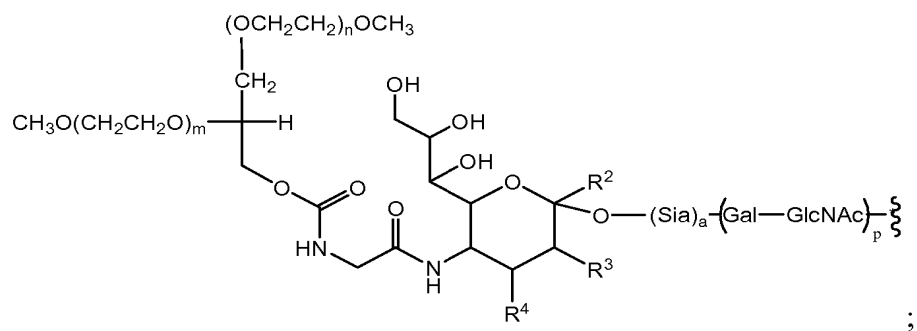
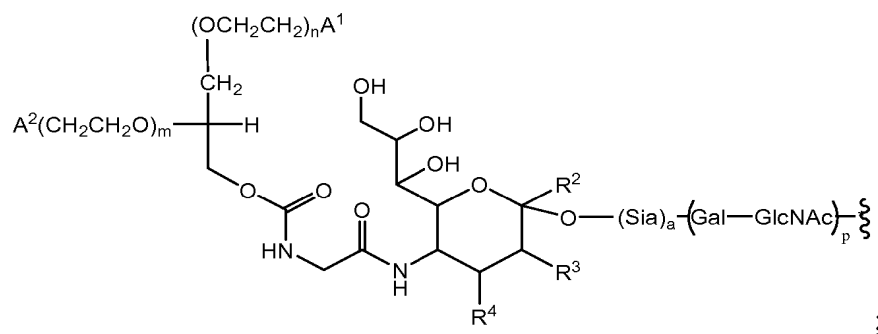


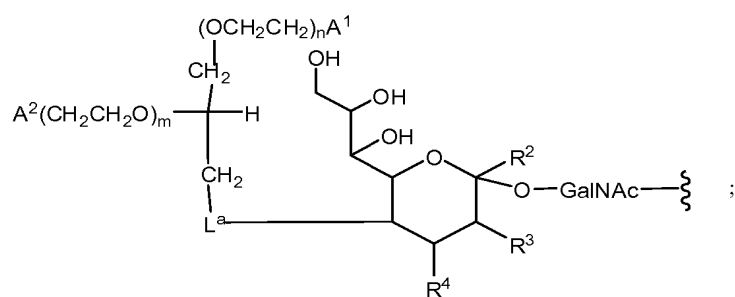
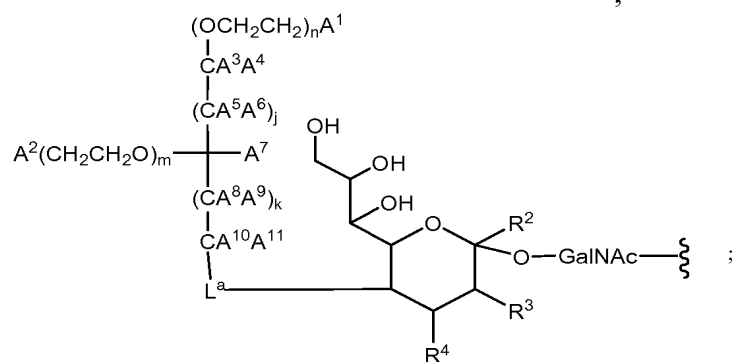
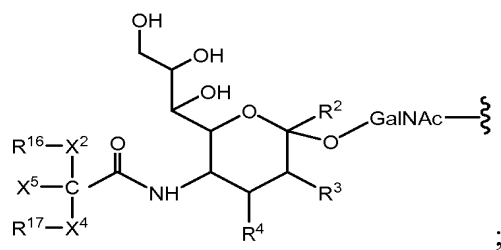
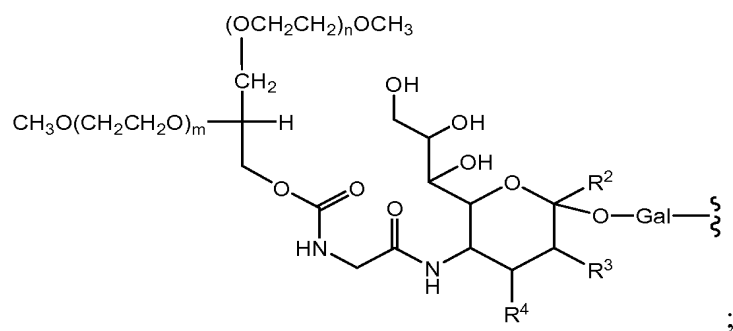
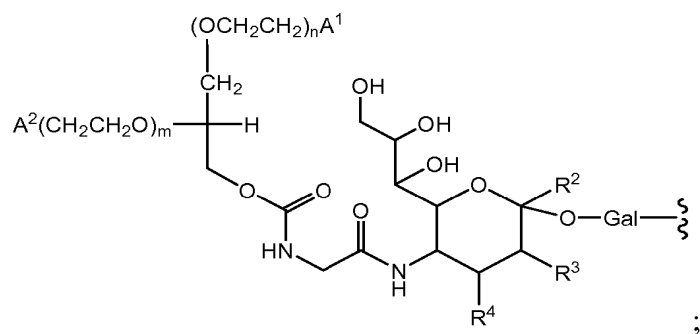




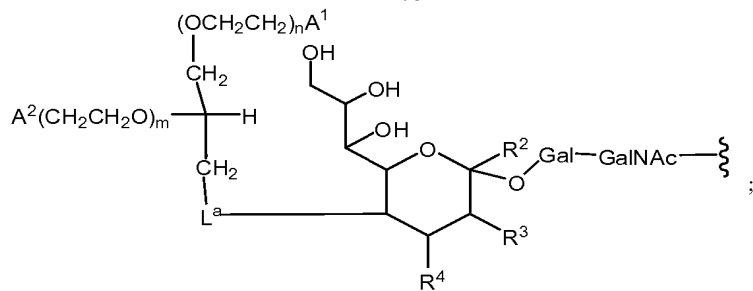
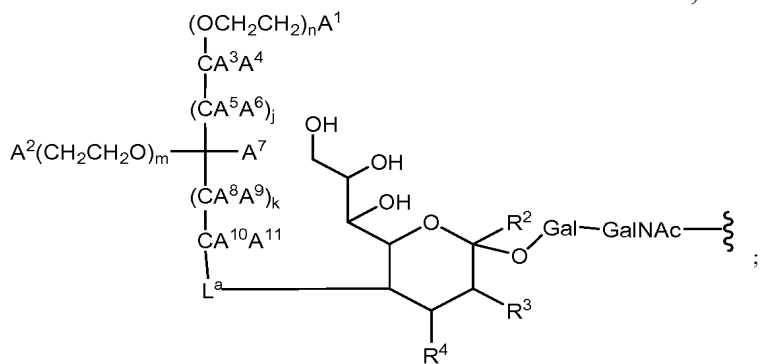
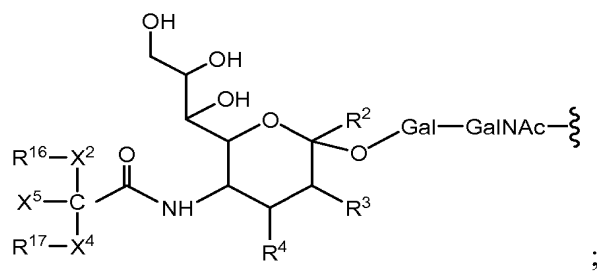
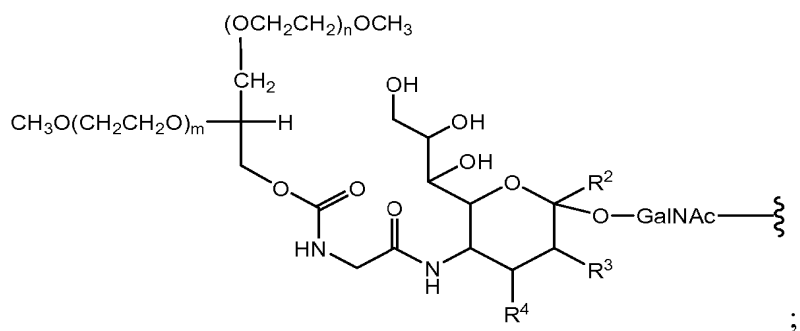
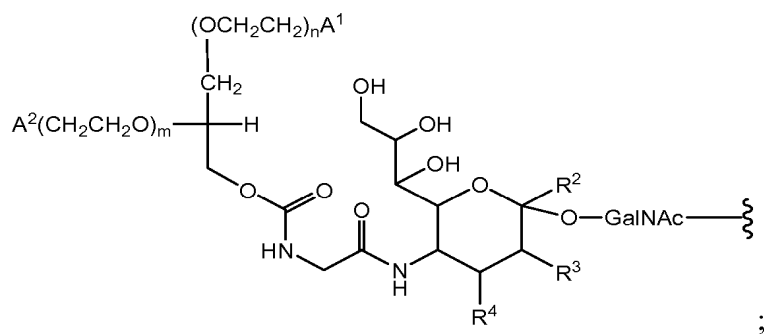


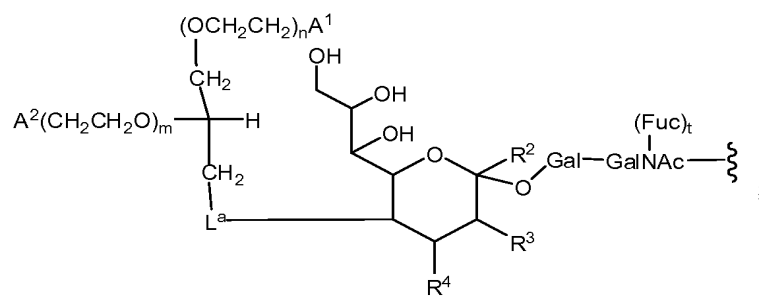
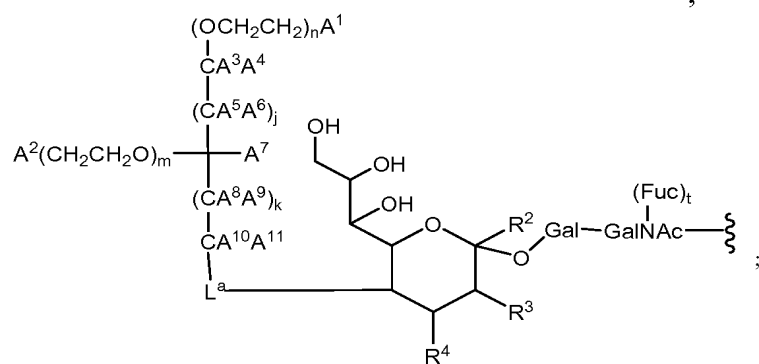
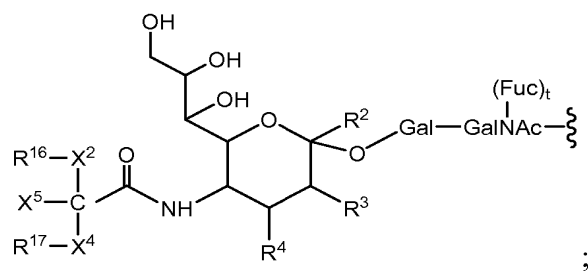
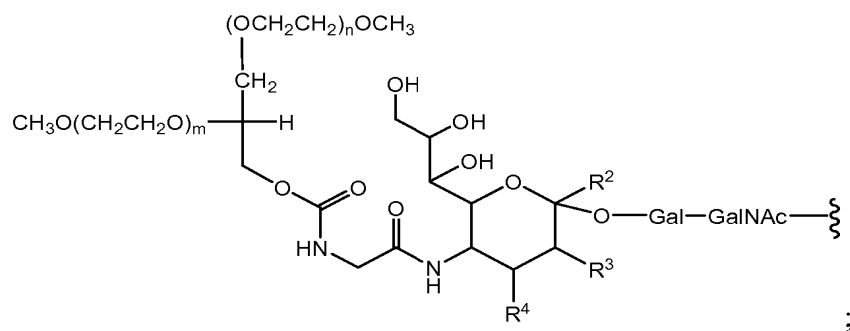
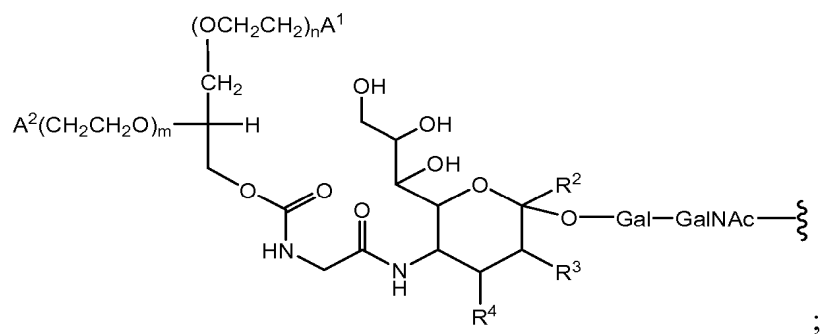


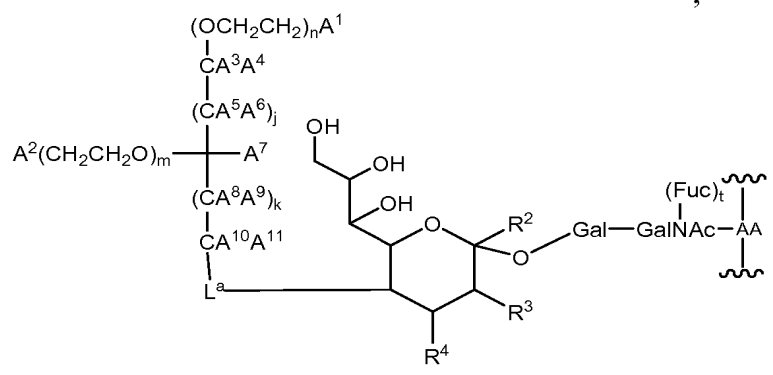
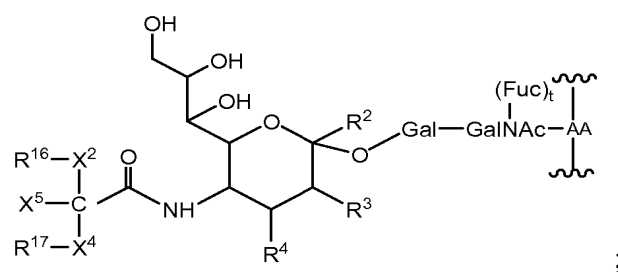
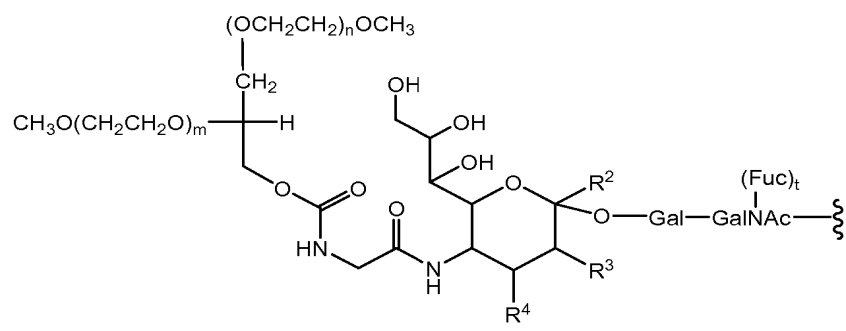
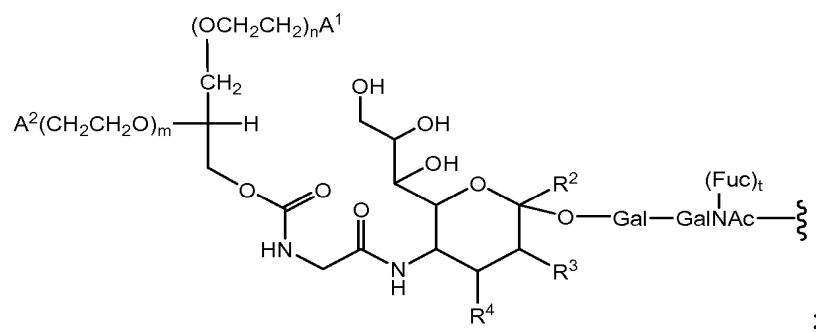




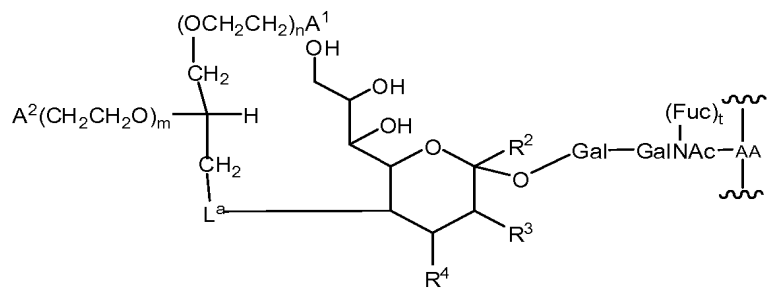


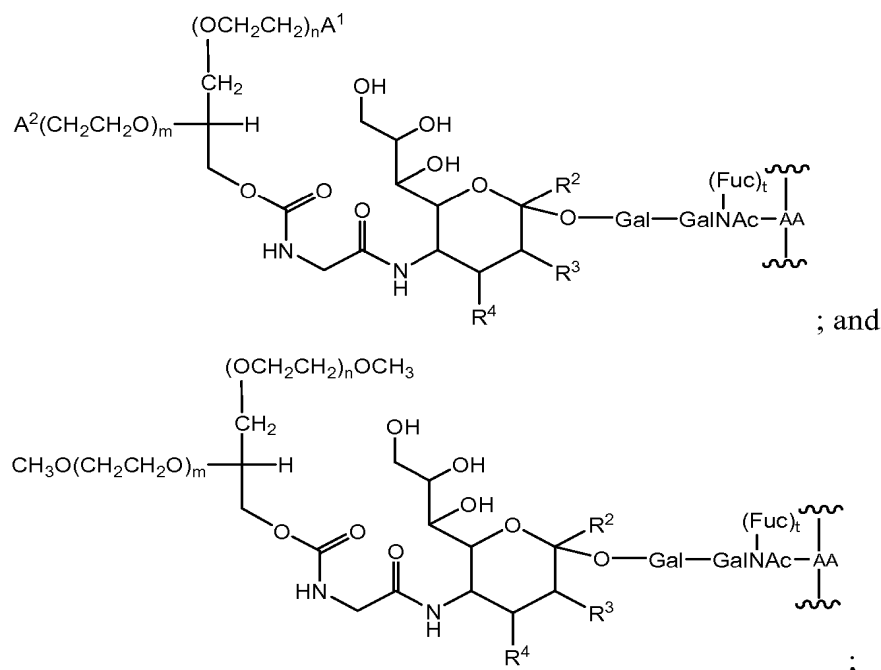






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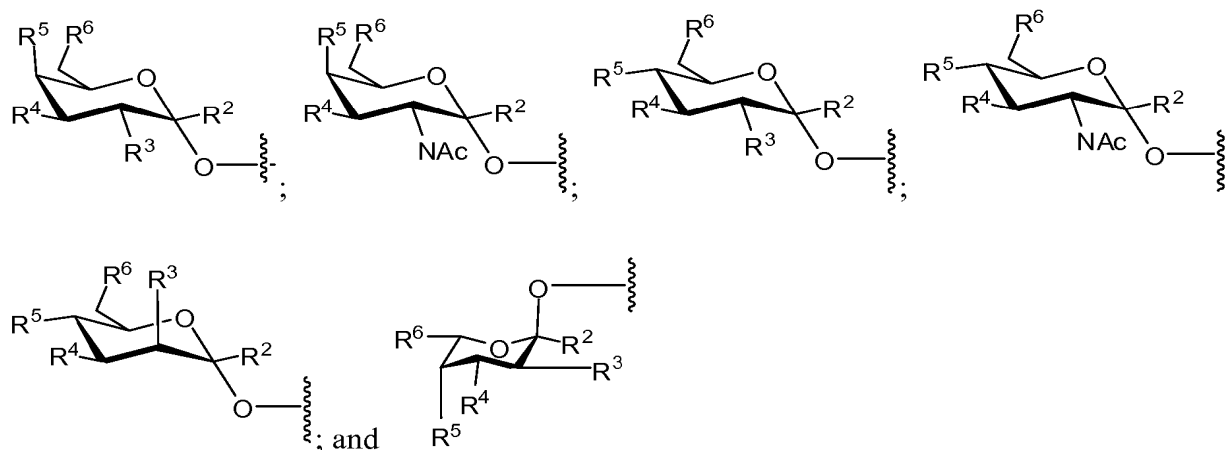
in which the index  $a$  and the linker  $L^a$  are as discussed above. The index  $p$  is an integer from 1 to 10. The indices  $t$  and  $a$  are independently selected from 0 or 1. Each of these groups can be included as components of the mono-, bi-, tri- and tetra-antennary saccharide structures set forth above. AA is an amino acid residue of the peptide. One of skill in the art will appreciate that the PEG moiety in these formulae can be replaced with other non-reactive group and polymeric moieties. Exemplary polymers include those of the poly(alkylene oxide) family. Non-reactive groups include groups that are considered to be essentially unreactive, neutral and/ or stable at physiological pH, e.g., H, substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl and the like. An exemplary polymeric moiety includes the branched structures set forth in Formula IIIa and its exemplars.

**[0146]** In an exemplary embodiment, the PEG moiety has a molecular weight of about 20 kD. In another exemplary embodiment, the PEG moiety has a molecular weight of about 5 kD. In another exemplary embodiment, the PEG moiety has a molecular weight of about 10 kD. In another exemplary embodiment, the PEG moiety has a molecular weight of about 40 kD. In other embodiments, the modifying group is a branched poly(alkylene oxide), e.g., poly(ethylene glycol), having a molecular weight of at least about 80 kD, preferably at least about 100 kD, more preferably at least about 120 kD, at least about 140 kD or at least about 160 kD. In yet another embodiment, the branched poly(alkylene oxide), e.g., poly(ethylene glycol) is at least about 200 kD, such as from at least about 80 kD to at least about 200 kD, including at least about 160 kD and at least about 180 kD. In an exemplary embodiment, the

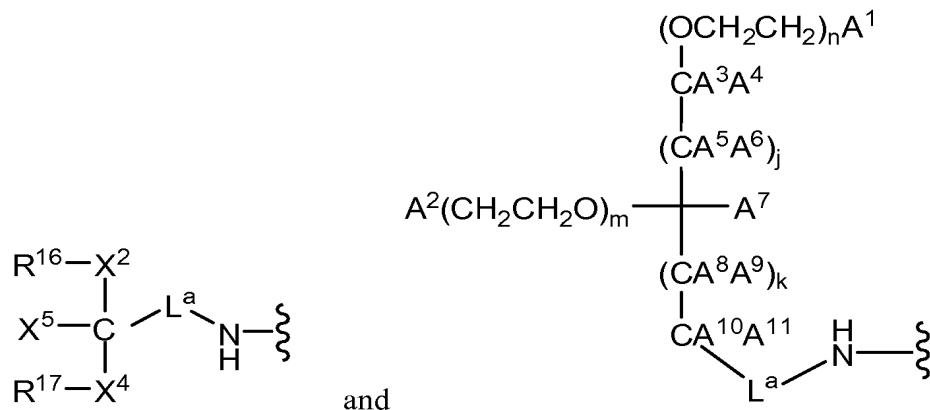
branched polymer is itself attached to a branching moiety (e.g., cysteine, serine, lysine, and oligomers of lysine).

[0147] In an exemplary embodiment, the glycosyl linking group is a branched SA-PEG-10 kD moiety based on a cysteine residue, and one or two of these glycosyl linking groups are covalently attached to the peptide. In another exemplary embodiment, the glycosyl linking group is a branched SA-PEG-10 kD moiety based on a lysine residue, and one or two of these glycosyl linking groups are covalently attached to the peptide. In an exemplary embodiment, the glycosyl linking group is a branched SA-PEG-10 kD moiety based on a cysteine residue, and one or two of these glycosyl linking groups are covalently attached to the peptide. In an exemplary embodiment, the glycosyl linking group is a branched SA-PEG-10 kD moiety based on a lysine residue, and one or two of these glycosyl linking groups are covalently attached to the peptide. In an exemplary embodiment, the glycosyl linking group is a branched SA-PEG-5 kD moiety based on a cysteine residue, and one, two or three of these glycosyl linking groups are covalently attached to the peptide. In an exemplary embodiment, the glycosyl linking group is a branched SA-PEG-5 kD moiety based on a lysine residue, and one, two or three of these glycosyl linking groups are covalently attached to the peptide. In an exemplary embodiment, the glycosyl linking group is a branched SA-PEG-40 kD moiety based on a cysteine residue, and one or two of these glycosyl linking groups are covalently attached to the peptide. In an exemplary embodiment, the glycosyl linking group is a branched SA-PEG-40 kD moiety based on a lysine residue, and one or two of these glycosyl linking groups are covalently attached to the peptide.

[0148] In another exemplary embodiment, the peptide conjugate comprises a glycosyl moiety selected from the formulae:

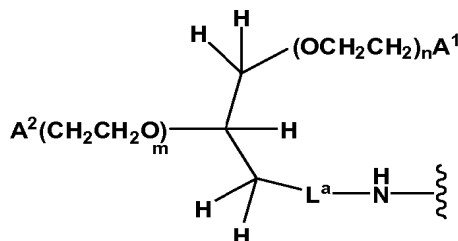


wherein at least one of  $R^2$ ,  $R^3$ ,  $R^4$ ,  $R^5$  or  $R^6$  has a structure which is a member selected from



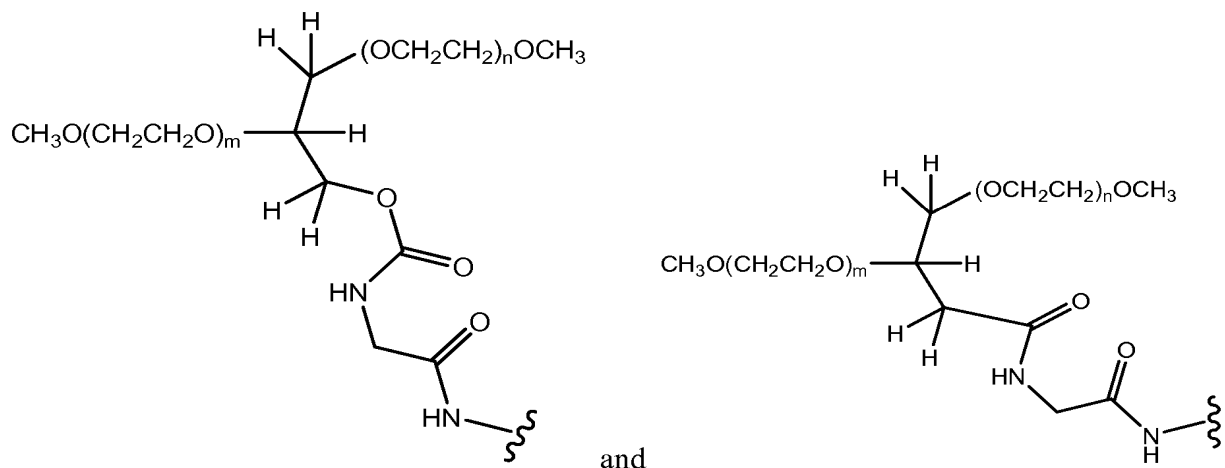
in which the variables are as described above. Those of skill will appreciate that the reliance on branched PEG structures set forth above is simply for clarity of illustration, the PEG can be replaced by substantially any polymeric moiety, including, without limitation those species set forth in the definition of “polymeric moiety” found herein.

[0149] In an exemplary embodiment, at least one of  $\text{R}^2$ ,  $\text{R}^3$ ,  $\text{R}^4$ ,  $\text{R}^5$  or  $\text{R}^6$  has a structure according to the following formula:



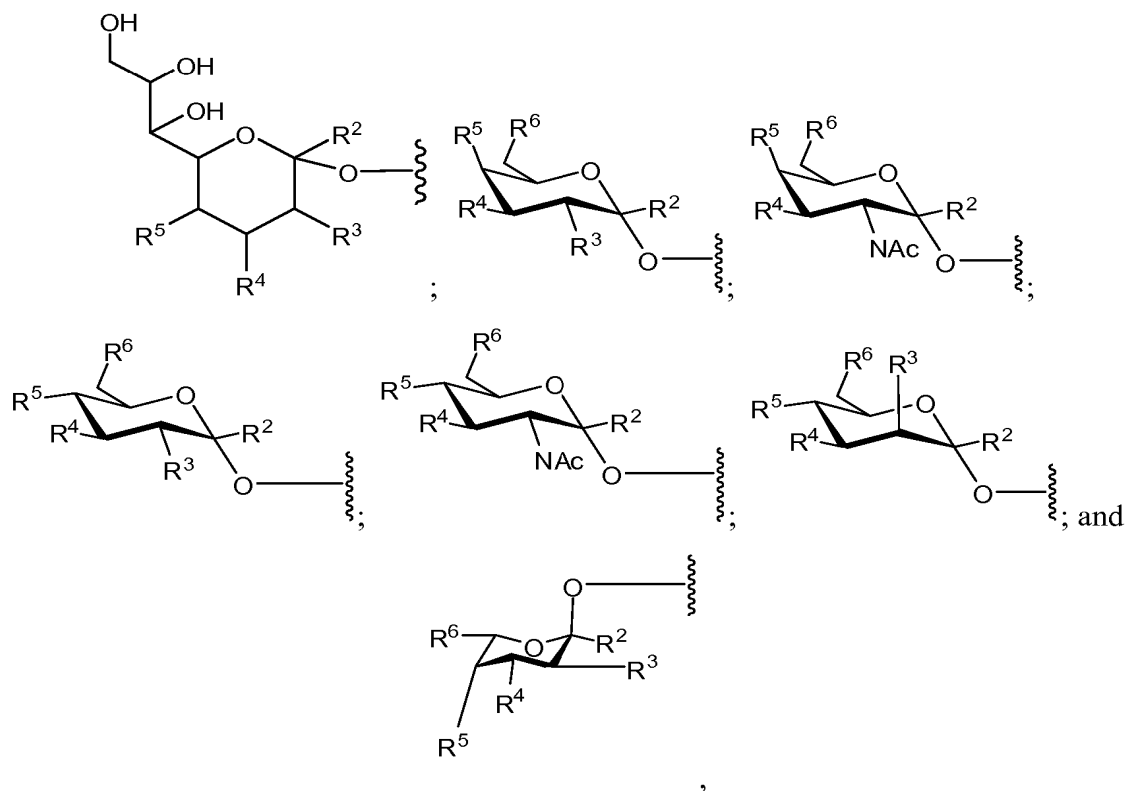
In an exemplary embodiment,  $\text{A}^1$  and  $\text{A}^2$  are each selected from  $-\text{OH}$  and  $-\text{OCH}_3$ .

10 [0150] Exemplary polymeric modifying groups according to this embodiment include:



[0151] In an exemplary embodiment, only one of  $\text{R}^2$ ,  $\text{R}^3$ ,  $\text{R}^4$ ,  $\text{R}^5$  or  $\text{R}^6$  has a structure which includes the modifying groups described above.

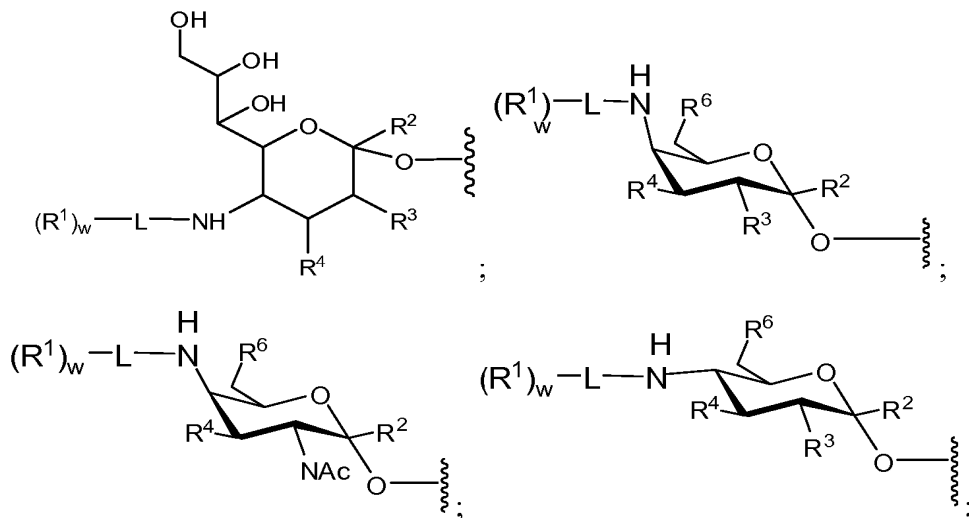
[0152] In another exemplary embodiment, the peptide conjugate comprises a glycosyl moiety selected from the formulae:



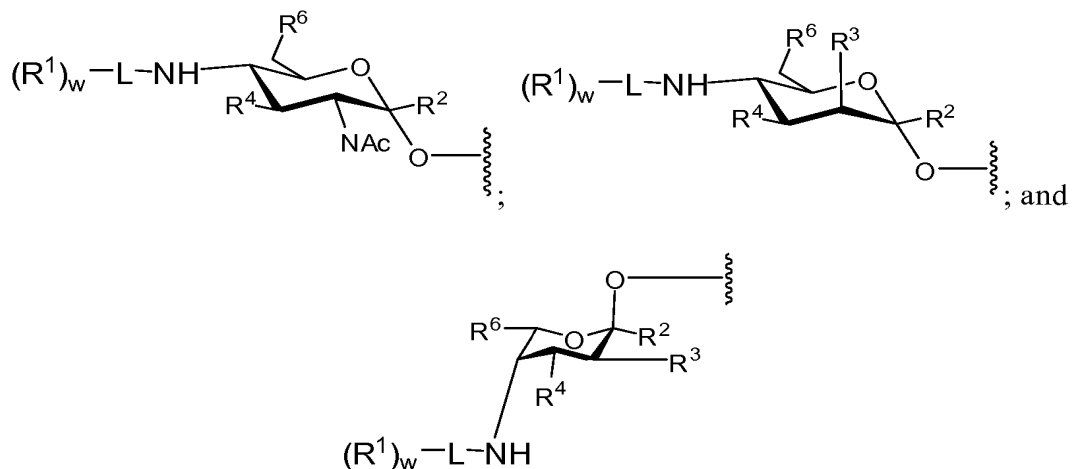
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wherein  $R^2$ ,  $R^3$ ,  $R^4$ ,  $R^5$  or  $R^6$  are as described above.

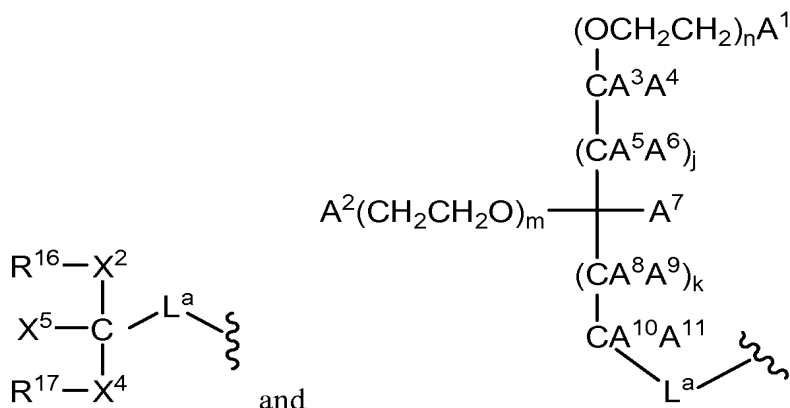
[0153] In another exemplary embodiment, the peptide conjugate comprises a glycosyl moiety selected from the formulae:



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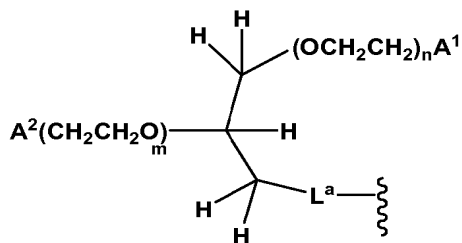


in which  $L-(R^1)_w$  is a member selected from



5 in which the variables are as described above.

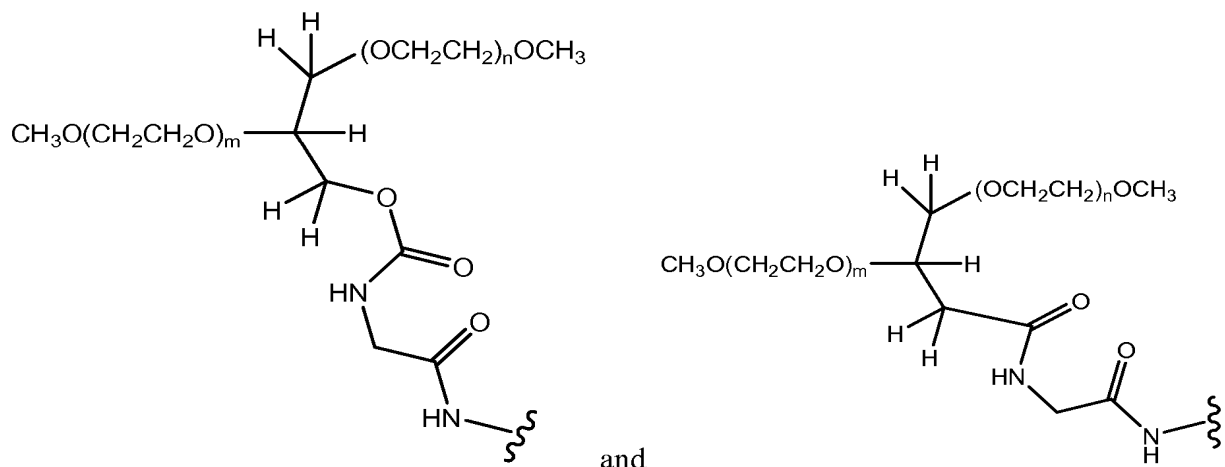
[0154] In an exemplary embodiment,  $L-(R^1)_w$  has a structure according to the following formula:



In an exemplary embodiment,  $A^1$  and  $A^2$  are each selected from  $-OH$  and  $-OCH_3$ .

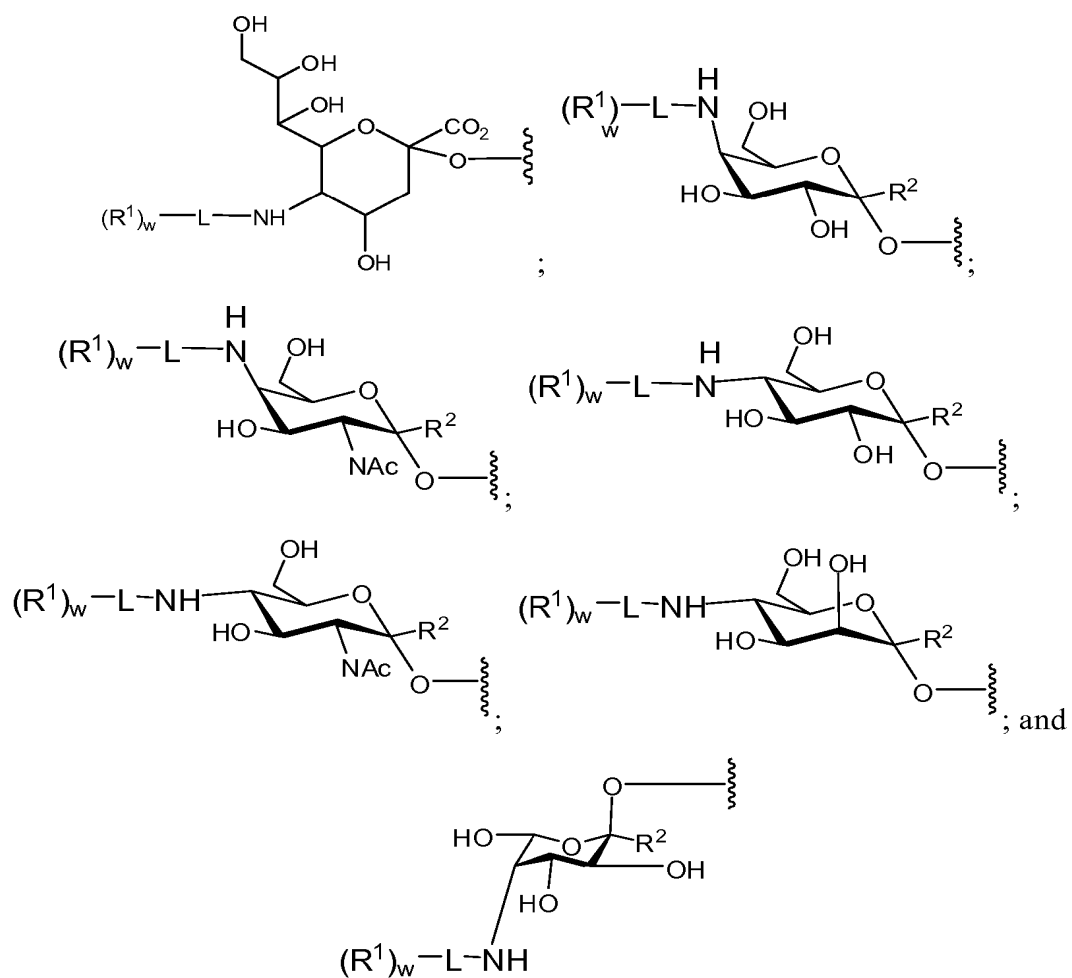


[0155] Exemplary polymeric modifying groups according to this embodiment include:



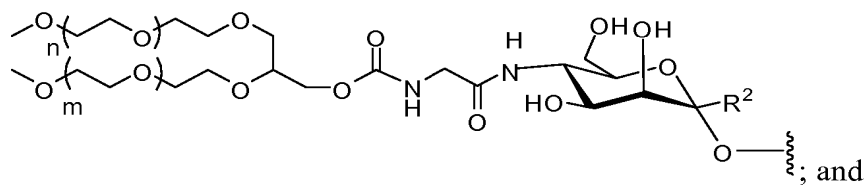
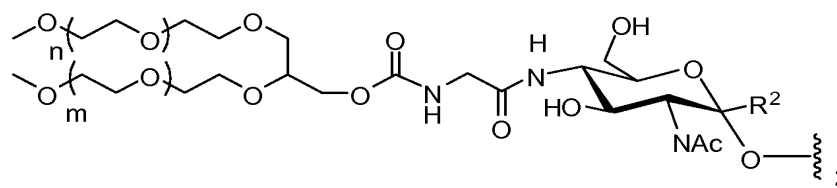
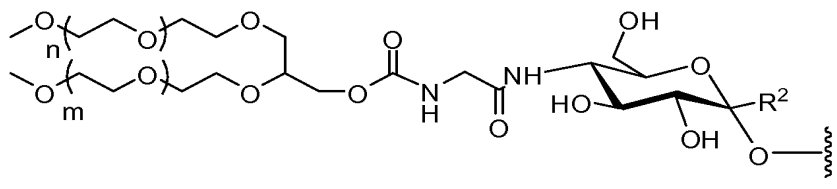
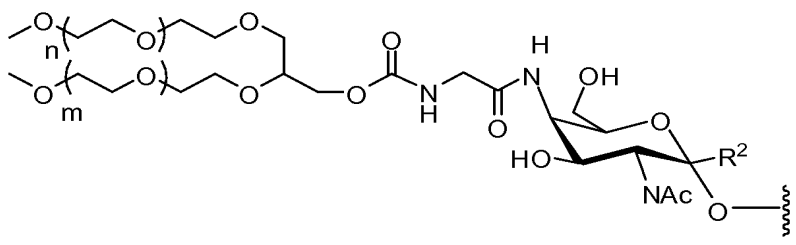
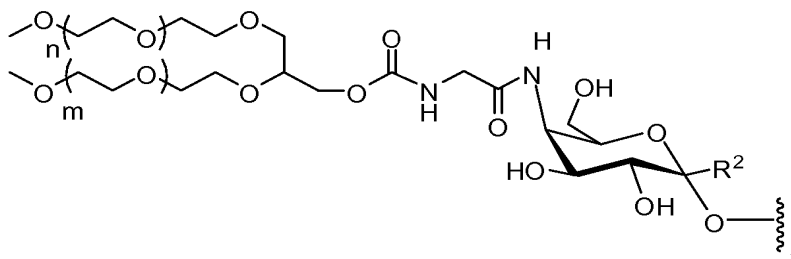
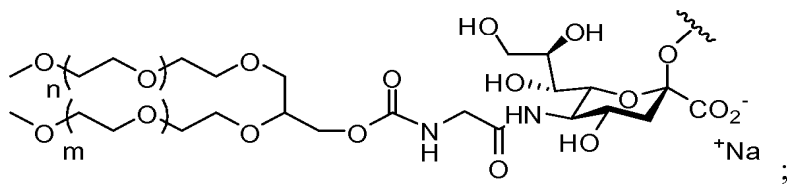
In an exemplary embodiment, m and n are integers independently selected from about 1 to about 1000. In an exemplary embodiment, m and n are integers independently selected from about 1 to about 500. In an exemplary embodiment, m and n are integers independently selected from about 1 to about 70, about 70 to about 150, about 150 to about 250, about 250 to about 375 and about 375 to about 500. In an exemplary embodiment, m and n are integers independently selected from about 10 to about 35, about 45 to about 65, about 95 to about 130, about 210 to about 240, about 310 to about 370 and about 420 to about 480. In an exemplary embodiment, m and n are integers selected from about 15 to about 30. In an exemplary embodiment, m and n are integers selected from about 50 to about 65. In an exemplary embodiment, m and n are integers selected from about 100 to about 130. In an exemplary embodiment, m and n are integers selected from about 210 to about 240. In an exemplary embodiment, m and n are integers selected from about 310 to about 370. In an exemplary embodiment, m and n are integers selected from about 430 to about 470.

[0156] In another exemplary embodiment, the peptide conjugate comprises a glycosyl moiety selected from the formulae:

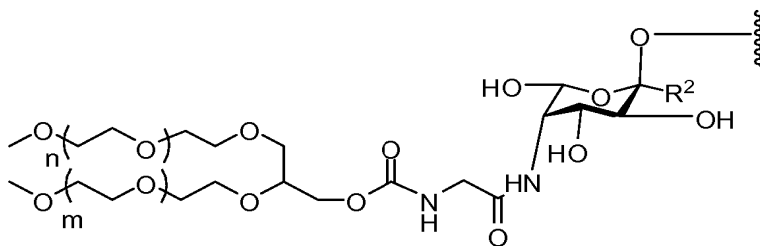


5 wherein the variables are as described above.

[0157] In another exemplary embodiment, species according to this embodiment include:

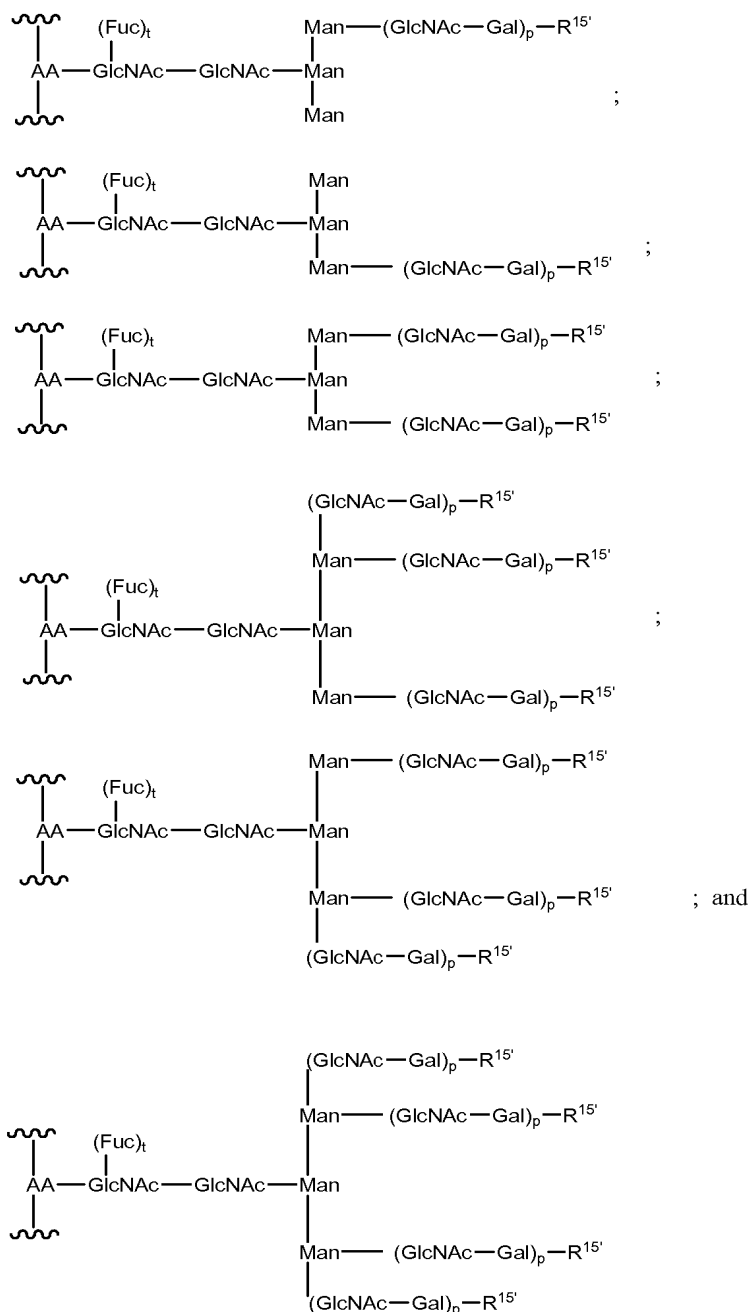


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wherein the variables are as discussed above.

**[0158]** In an exemplary embodiment, a glycoPEGylated peptide conjugate of the invention is selected from the formulae set forth below:



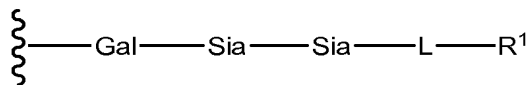
wherein the variables are as described above.

[0159] In the formulae above, the index  $t$  is an integer from 0 to 1 and the index  $p$  is an integer from 1 to 10. The symbol  $\text{R}^{15'}$  represents H, OH (e.g., Gal-OH), a sialyl moiety, a sialyl linking group (i.e., sialyl linking group-polymeric modifying group ( $\text{Sia-L-R}^1$ ), or a sialyl moiety to which is bound a polymer modified sialyl moiety (e.g.,  $\text{Sia-Sia-L-R}^1$ ) (“Sia-Sia<sup>P</sup>”), a galactosyl moiety, a galactosyl linking group (i.e., galactosyl linking group-polymeric modifying group ( $\text{Gal-L-R}^1$ ), or a sialyl moiety to which is bound a polymer

modified galactosyl moiety (e.g., Sia-Gal-L-R<sup>1</sup>) ("Sia-Gal<sup>P</sup>"), a galactosaminyl moiety, a galactosaminyl linking group (i.e., galactosaminyl linking group-polymeric modifying group (GalNAc-L-R<sup>1</sup>), or a sialyl moiety to which is bound a polymer modified galactosaminyl moiety (e.g., Sia-GalNAc-L-R<sup>1</sup>) ("Sia-GalNAc<sup>P</sup>"), a glucosyl moiety, a glucosyl linking group (i.e., glucosyl linking group-polymeric modifying group (Glc-L-R<sup>1</sup>), or a sialyl moiety to which is bound a polymer modified glucosyl moiety (e.g., Sia-Glc-L-R<sup>1</sup>) ("Sia-Glc<sup>P</sup>"), a glucosaminyl moiety, a glucosaminyl linking group (i.e., glucosaminyl linking group-polymeric modifying group (GlcNAc-L-R<sup>1</sup>), or a sialyl moiety to which is bound a polymer modified glucosaminyl moiety (e.g., Sia-GlcNAc-L-R<sup>1</sup>) ("Sia-GlcNAc<sup>P</sup>"), a mannosyl moiety, a mannosyl linking group (i.e., mannosyl linking group-polymeric modifying group (Man-L-R<sup>1</sup>), or a sialyl moiety to which is bound a polymer modified mannosyl moiety (e.g., Sia-Man-L-R<sup>1</sup>) ("Sia-Man<sup>P</sup>"), a fucosyl moiety, a fucosyl linking group (i.e., fucosyl linking group-polymeric modifying group (Fuc-L-R<sup>1</sup>), or a sialyl moiety to which is bound a polymer modified fucosyl moiety (e.g., Sia-Fuc-L-R<sup>1</sup>) ("Sia-Fuc<sup>P</sup>").

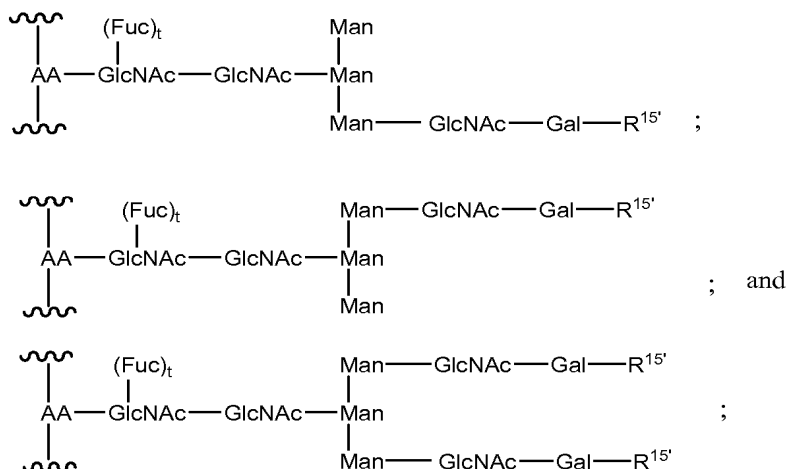
Exemplary polymer modified saccharyl moieties have a structure according to Formulae I, Ia, II or IIa. An exemplary peptide conjugate of the invention will include at least one glycan having a R<sup>15'</sup> that includes a structure according to Formulae I, Ia, II and IIa. The oxygen, with the open valence, of Formulae I, Ia, II or IIa is preferably attached through a glycosidic linkage to a carbon of a Gal or GalNAc moiety. In a further exemplary embodiment, the oxygen is attached to the carbon at position 3 of a galactose residue. In an exemplary embodiment, the modified sialic acid is linked α2,3-to the galactose residue. In another exemplary embodiment, the sialic acid is linked α2,6-to the galactose residue.

**[0160]** In an exemplary embodiment, the sialyl linking group is a sialyl moiety to which is bound a polymer modified sialyl moiety (e.g., Sia-Sia-L-R<sup>1</sup>) ("Sia-Sia<sup>P</sup>"). Here, the glycosyl linking group is linked to a galactosyl moiety through a sialyl moiety:



An exemplary species according to this motif is prepared by conjugating Sia-L-R<sup>1</sup> to a terminal sialic acid of a glycan using an enzyme that forms Sia-Sia bonds, e.g., CST-II, ST8Sia-II, ST8Sia-III and ST8Sia-IV.

**[0161]** In another exemplary embodiment, the glycans on the peptide conjugates have a formula that is selected from the group:



and combinations thereof.

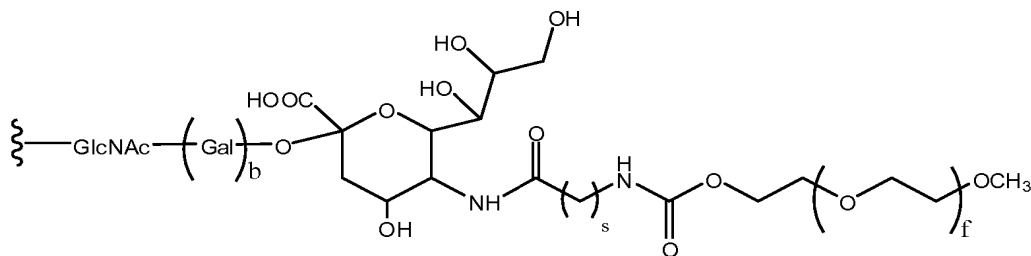
[0162] In each of the formulae above,  $\text{R}^{15'}$  is as discussed above. Moreover, an exemplary peptide conjugate of the invention will include at least one glycan with an  $\text{R}^{15}$  moiety having a structure according to Formulae I, Ia, II or IIa.

[0163] In another exemplary embodiment, the glycosyl linking group comprises at least one glycosyl linking group having the formula:



wherein  $\text{R}^{15}$  is said sialyl linking group; and the index  $p$  is an integer selected from 1 to 10.

[0164] In an exemplary embodiment, the glycosyl linking moiety has the formula:



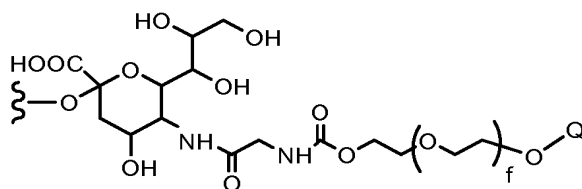
in which  $b$  is an integer from 0 to 1. The index  $s$  represents an integer from 1 to 10; and the index  $f$  represents an integer from 1 to 2500.

[0165] In an exemplary embodiment, the polymeric modifying group is PEG. In another exemplary embodiment, the PEG moiety has a molecular weight of about 20 kD. In another exemplary embodiment, the PEG moiety has a molecular weight of about 5 kD. In another exemplary embodiment, the PEG moiety has a molecular weight of about 10 kD. In another exemplary embodiment, the PEG moiety has a molecular weight of about 40 kD. In other

embodiments, the modifying group is a branched poly(alkylene oxide), e.g., poly(ethylene glycol), having a molecular weight of at least about 80 kD, preferably at least about 100 kD, more preferably at least about 120 kD, at least about 140 kD or at least about 160 kD. In yet another embodiment, the branched poly(alkylene oxide), e.g., poly(ethylene glycol) is at least about 200 kD, such as from at least about 80 kD to at least about 200 kD, including at least about 160 kD and at least about 180 kD.

[0166] In an exemplary embodiment, the glycosyl linking group is a linear SA-PEG-10 kD moiety, and one or two of these glycosyl linking groups are covalently attached to the peptide. In another exemplary embodiment, the glycosyl linking group is a linear SA-PEG-20 kD moiety, and one or two of these glycosyl linking groups are covalently attached to the peptide. In an exemplary embodiment, the glycosyl linking group is a linear SA-PEG-5 kD moiety, and one, two or three of these glycosyl linking groups are covalently attached to the peptide. In an exemplary embodiment, the glycosyl linking group is a linear SA-PEG-40 kD moiety, and one or two of these glycosyl linking groups are covalently attached to the peptide.

[0167] In another exemplary embodiment, the glycosyl linking group is a sialyl linking group having the formula:

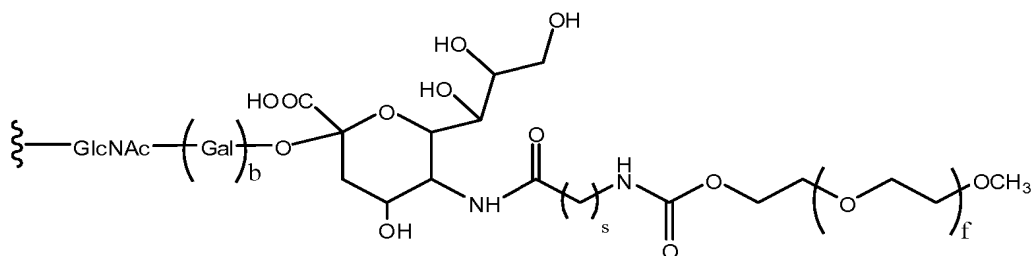


In another exemplary embodiment, Q is a member selected from H and CH<sub>3</sub>. In another exemplary embodiment, wherein said glycosyl linking group has the formula:



wherein R<sup>15</sup> is said sialyl linking group; and the index p is an integer selected from 1 to 10.

In an exemplary embodiment, the glycosyl linking group comprises the formula:

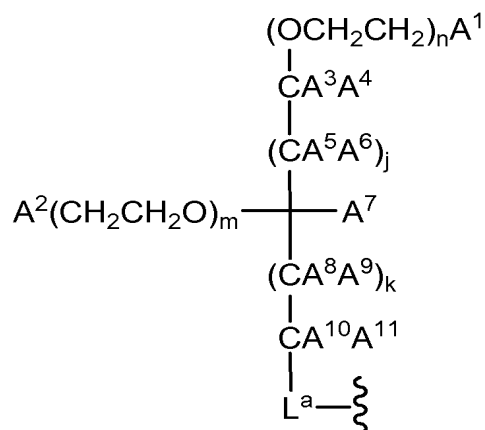




wherein the index b is an integer selected from 0 and 1. In an exemplary embodiment, the index s is 1; and the index f is an integer selected from about 200 to about 300.

## II. D. Modifying Groups

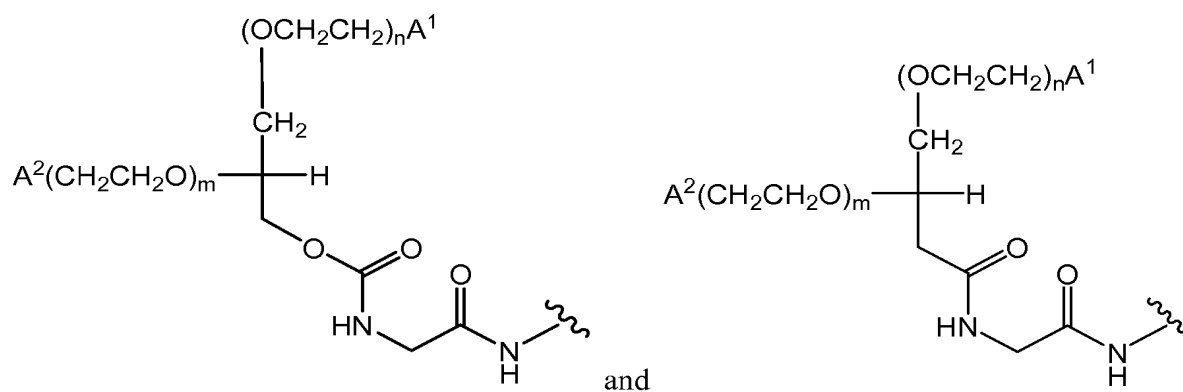
[0168] The peptide conjugates of the invention comprise a modifying group. This group can be covalently attached to a peptide through an amino acid or a glycosyl linking group. In another exemplary embodiment, when the modifying group includes the moiety:



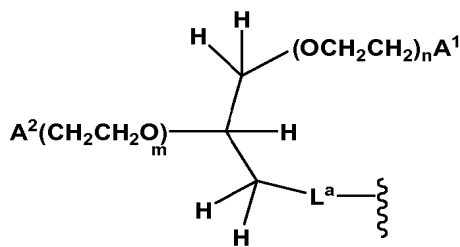
and the peptide in the peptide conjugate is a member selected from the peptides in **FIG. 7**. In another exemplary embodiment, the peptide in the peptide conjugate is a member selected from bone morphogenetic proteins (e.g., BMP-1, BMP-2, BMP-3, BMP-4, BMP-5, BMP-6, BMP-7, BMP-8, BMP-9, BMP-10, BMP-11, BMP-12, BMP-13, BMP-14, BMP-15), neurotrophins (e.g., NT-3, NT-4, NT-5), growth differentiation factors (e.g., GDF-5), glial cell line-derived neurotrophic factor (GDNF), brain derived neurotrophic factor (BDNF), nerve growth factor (NGF), von Willebrand factor (vWF) protease, Factor VII, Factor VIIa, Factor VIII, Factor IX, Factor X, Factor XI, B-domain deleted Factor VIII, vWF-Factor VIII fusion protein having full-length Factor VIII, vWF-Factor VIII fusion protein having B-domain deleted Factor VIII, erythropoietin (EPO), granulocyte colony stimulating factor (G-CSF), Granulocyte-Macrophage Colony Stimulating Factor (GM-CSF) interferon alpha, interferon beta, interferon gamma,  $\alpha_1$ -antitrypsin (ATT, or  $\alpha$ -1 protease inhibitor), glucocerebrosidase, Tissue-Type Plasminogen Activator (TPA), Interleukin-2 (IL-2), urokinase, human DNase, insulin, Hepatitis B surface protein (HbsAg), human growth hormone, TNF Receptor-IgG Fc region fusion protein (Enbrel™), anti-HER2 monoclonal antibody (Herceptin™), monoclonal antibody to Protein F of Respiratory Syncytial Virus (Synagis™), monoclonal antibody to TNF- $\alpha$  (Remicade™), monoclonal antibody to glycoprotein IIb/IIIa (Reopro™), monoclonal antibody to CD20 (Rituxan™), anti-thrombin III (AT III), human Chorionic Gonadotropin (hCG), alpha-galactosidase (Fabrazyme™),

alpha-iduronidase (Aldurazyme™), follicle stimulating hormone, beta-glucosidase, anti-TNF-alpha monoclonal antibody, glucagon-like peptide-1 (GLP-1), glucagon-like peptide-2 (GLP-2), beta-glucosidase, alpha-galactosidase A and fibroblast growth factor. “Modifying groups” can encompass a variety of structures including targeting moieties, therapeutic moieties, biomolecules. Additionally, “modifying groups” include polymeric modifying groups, which are polymers which can alter a property of the peptide such as its bioavailability or its half-life in the body.

[0169] In an exemplary embodiment, the polymeric modifying group has a structure including a moiety according to the following formulae:

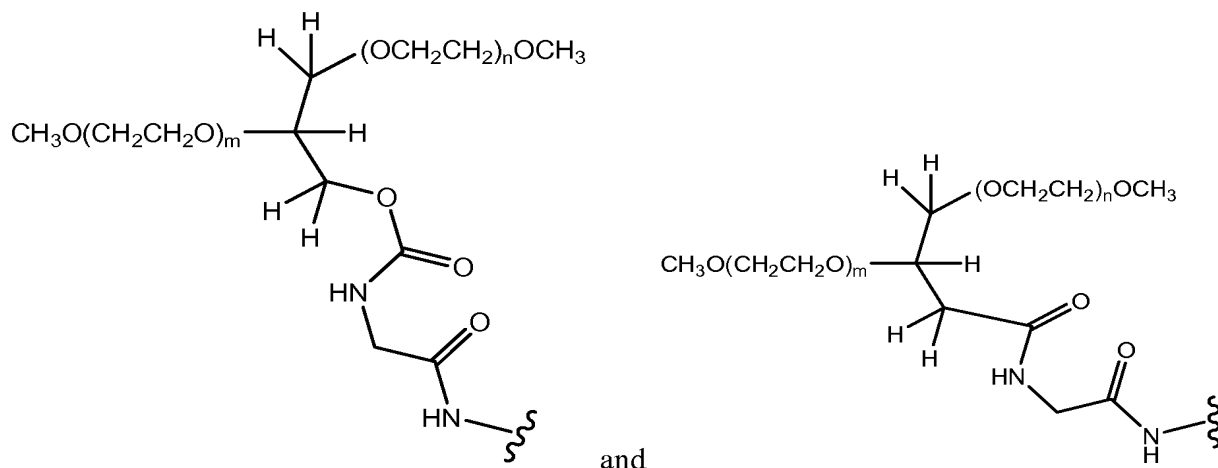


[0170] In another exemplary embodiment according to the formula above, the polymeric modifying group includes a moiety according to the following formula:



In an exemplary embodiment, A¹ and A² are each members selected from -OH and -OCH₃.

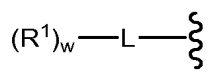
[0171] Exemplary polymeric modifying groups according to this embodiment include the moiety:



[0172] For the purposes of convenience, the modifying groups in the remainder of this section will be largely based on polymeric modifying groups such as water soluble and water insoluble polymers. However, one of skill in the art will recognize that other modifying groups, such as targeting moieties, therapeutic moieties and biomolecules, could be used in place of the polymeric modifying groups. In addition, those of skill will appreciate that the reliance on branched PEG structures set forth above is simply for clarity of illustration, the PEG can be replaced by substantially any polymeric moiety, including, without limitation those species set forth in the definition of “polymeric moiety” found herein.

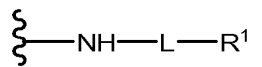
#### ***II. D. i. Linkers of the Modifying Groups***

[0173] The linkers of the modifying group serve to attach the modifying group (ie polymeric modifying groups, targeting moieties, therapeutic moieties and biomolecules) to the peptide. In an exemplary embodiment, the polymeric modifying group is bound to a glycosyl linking group, generally through a heteroatom, e.g, nitrogen, on the core through a linker, L, as shown below:

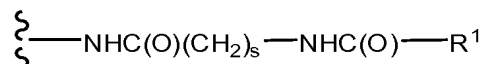


$R^1$  is the polymeric moiety and L is selected from a bond and a linking group. The index w represents an integer selected from 1-6, preferably 1-3 and more preferably 1-2. Exemplary linking groups include substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl moieties and sialic acid. An exemplary component of the linker is an acyl moiety.

[0174] An exemplary compound according to the invention has a structure according to Formulae I, Ia, II or IIa above, in which at least one of  $R^2$ ,  $R^3$ ,  $R^4$ ,  $R^5$ ,  $R^6$  or  $R^{6'}$  has the formula:

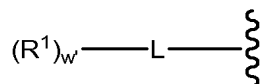


5 [0175] In another example according to this embodiment at least one of  $R^2$ ,  $R^3$ ,  $R^4$ ,  $R^5$ ,  $R^6$  or  $R^{6'}$  has the formula:



in which  $s$  is an integer from 0 to 20 and  $R^1$  is a linear polymeric modifying moiety.

10 [0176] In an exemplary embodiment, the polymeric modifying group -linker construct is a branched structure that includes two or more polymeric chains attached to central moiety. In this embodiment, the construct has the formula:



in which  $R^1$  and  $L$  are as discussed above and  $w$  is an integer from 2 to 6, preferably from 2 to 4 and more preferably from 2 to 3.

15 [0177] When  $L$  is a bond it is formed between a reactive functional group on a precursor of  $R^1$  and a reactive functional group of complementary reactivity on the saccharyl core. When  $L$  is a non-zero order linker, a precursor of  $L$  can be in place on the glycosyl moiety prior to reaction with the  $R^1$  precursor. Alternatively, the precursors of  $R^1$  and  $L$  can be incorporated into a preformed cassette that is subsequently attached to the glycosyl moiety. As set forth  
20 herein, the selection and preparation of precursors with appropriate reactive functional groups is within the ability of those skilled in the art. Moreover, coupling the precursors proceeds by chemistry that is well understood in the art.

[0178] In an exemplary embodiment,  $L$  is a linking group that is formed from an amino acid, or small peptide (e.g., 1-4 amino acid residues) providing a modified sugar in which the  
25 polymeric modifying group is attached through a substituted alkyl linker. Exemplary linkers include glycine, lysine, serine and cysteine. The PEG moiety can be attached to the amine moiety of the linker through an amide or urethane bond. The PEG is linked to the sulfur or oxygen atoms of cysteine and serine through thioether or ether bonds, respectively.

[0179] In an exemplary embodiment,  $R^5$  includes the polymeric modifying group. In  
30 another exemplary embodiment,  $R^5$  includes both the polymeric modifying group and a

linker, L, joining the modifying group to the remainder of the molecule. As discussed above, L can be a linear or branched structure. Similarly, the polymeric modifying group can be branched or linear.

#### ***II. D. ii. Water-Soluble Polymers***

5 [0180] Many water-soluble polymers are known to those of skill in the art and are useful in practicing the present invention. The term water-soluble polymer encompasses species such as saccharides (e.g., dextran, amylose, hyalouronic acid, poly(sialic acid), heparans, heparins, etc.); poly (amino acids), e.g., poly(aspartic acid) and poly(glutamic acid); nucleic acids; synthetic polymers (e.g., poly(acrylic acid), poly(ethers), e.g., poly(ethylene glycol);  
10 peptides, proteins, and the like. The present invention may be practiced with any water-soluble polymer with the sole limitation that the polymer must include a point at which the remainder of the conjugate can be attached.

[0181] Methods for activation of polymers can also be found in WO 94/17039, U.S. Pat. No. 5,324,844, WO 94/18247, WO 94/04193, U.S. Pat. No. 5,219,564, U.S. Pat. No.  
15 5,122,614, WO 90/13540, U.S. Pat. No. 5,281,698, and more WO 93/15189, and for conjugation between activated polymers and peptides, *e.g.* Coagulation Factor VIII (WO 94/15625), hemoglobin (WO 94/09027), oxygen carrying molecule (U.S. Pat. No. 4,412,989), ribonuclease and superoxide dismutase (Veronese *et al.*, *App. Biochem. Biotech.* **11**: 141-45 (1985)).

20 [0182] Exemplary water-soluble polymers are those in which a substantial proportion of the polymer molecules in a sample of the polymer are of approximately the same molecular weight; such polymers are “homodisperse.”

[0183] The present invention is further illustrated by reference to a poly(ethylene glycol) conjugate. Several reviews and monographs on the functionalization and conjugation of PEG  
25 are available. *See*, for example, Harris, *Macromol. Chem. Phys.* **C25**: 325-373 (1985); Scouten, *Methods in Enzymology* **135**: 30-65 (1987); Wong *et al.*, *Enzyme Microb. Technol.* **14**: 866-874 (1992); Delgado *et al.*, *Critical Reviews in Therapeutic Drug Carrier Systems* **9**: 249-304 (1992); Zalipsky, *Bioconjugate Chem.* **6**: 150-165 (1995); and Bhadra, *et al.*, *Pharmazie*, **57**:5-29 (2002). Routes for preparing reactive PEG molecules and forming  
30 conjugates using the reactive molecules are known in the art. For example, U.S. Patent No. 5,672,662 discloses a water soluble and isolatable conjugate of an active ester of a polymer

acid selected from linear or branched poly(alkylene oxides), poly(oxyethylated polyols), poly(olefinic alcohols), and poly(acrylomorpholine).

[0184] U.S. Patent No. 6,376,604 sets forth a method for preparing a water-soluble 1-benzotriazolylcarbonate ester of a water-soluble and non-peptidic polymer by reacting a terminal hydroxyl of the polymer with di(1-benzotriazolyl)carbonate in an organic solvent. The active ester is used to form conjugates with a biologically active agent such as a protein or peptide.

[0185] WO 99/45964 describes a conjugate comprising a biologically active agent and an activated water soluble polymer comprising a polymer backbone having at least one terminus linked to the polymer backbone through a stable linkage, wherein at least one terminus comprises a branching moiety having proximal reactive groups linked to the branching moiety, in which the biologically active agent is linked to at least one of the proximal reactive groups. Other branched poly(ethylene glycols) are described in WO 96/21469, U.S. Patent No. 5,932,462 describes a conjugate formed with a branched PEG molecule that includes a branched terminus that includes reactive functional groups. The free reactive groups are available to react with a biologically active species, such as a protein or peptide, forming conjugates between the poly(ethylene glycol) and the biologically active species. U.S. Patent No. 5,446,090 describes a bifunctional PEG linker and its use in forming conjugates having a peptide at each of the PEG linker termini.

[0186] Conjugates that include degradable PEG linkages are described in WO 99/34833; and WO 99/14259, as well as in U.S. Patent No. 6,348,558. Such degradable linkages are applicable in the present invention.

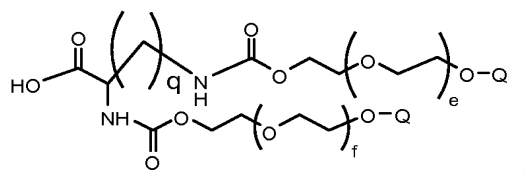
[0187] The art-recognized methods of polymer activation set forth above are of use in the context of the present invention in the formation of the branched polymers set forth herein and also for the conjugation of these branched polymers to other species, e.g., sugars, sugar nucleotides and the like.

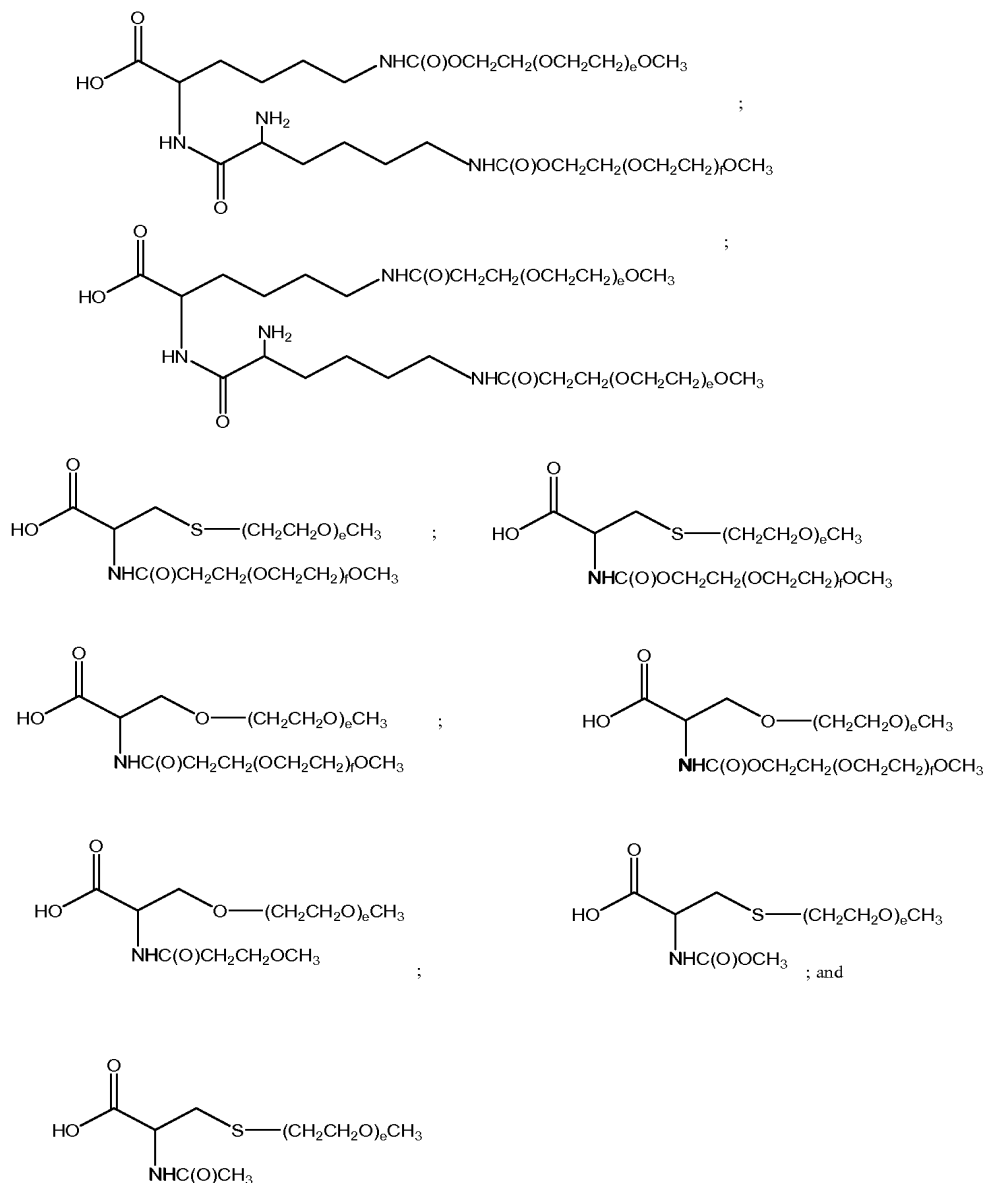
[0188] An exemplary water-soluble polymer is poly(ethylene glycol), e.g., methoxy-poly(ethylene glycol). The poly(ethylene glycol) used in the present invention is not restricted to any particular form or molecular weight range. For unbranched poly(ethylene glycol) molecules the molecular weight is preferably between 500 and 100,000. A molecular weight of 2000-60,000 is preferably used and preferably of from about 5,000 to about 40,000.

**II. D. iii. Branched Water Soluble Polymers**

[0189] In another embodiment the polymeric modifying moiety is a branched PEG structure having more than one linear or branched PEG moieties attached. Examples of branched PEGs are described in U.S. Pat. No. 5,932,462; U.S. Pat. No. 5,342,940; U.S. Pat. No. 5,643,575; U.S. Pat. No. 5,919,455; U.S. Pat. No. 6,113,906; U.S. Pat. No. 5,183,660; WO 02/09766; Kodera Y., *Bioconjugate Chemistry* **5**: 283-288 (1994); and Yamasaki et al., *Agric. Biol. Chem.*, **52**: 2125-2127, 1998.

[0190] Representative polymeric modifying moieties include structures that are based on side chain-containing amino acids, e.g., serine, cysteine, lysine, and small peptides, e.g., lys-lys. In some embodiments, the polymeric modifying moiety is a branched PEG moiety that is based upon an oligo-peptide, such as a tri-lysine peptide. Exemplary amino acids suitable for use include lysine, cysteine, and serine. In such embodiments, each polymeric subunit attached to the peptide structure may be either a linear PEG moiety or a branched PEG moiety. For example, the tri-lysine can be mono-, di-, tri-, or tetra-PEG-ylated with linear PEG moieties, branched PEG moieties, or a combination of linear and branched PEG moieties. Exemplary branched structures include the following moieties:





Those of skill will appreciate that the free amine in the di-lysine structures can also be pegylated through an amide or urethane bond with a either a linear PEG moiety or a branched PEG moiety.

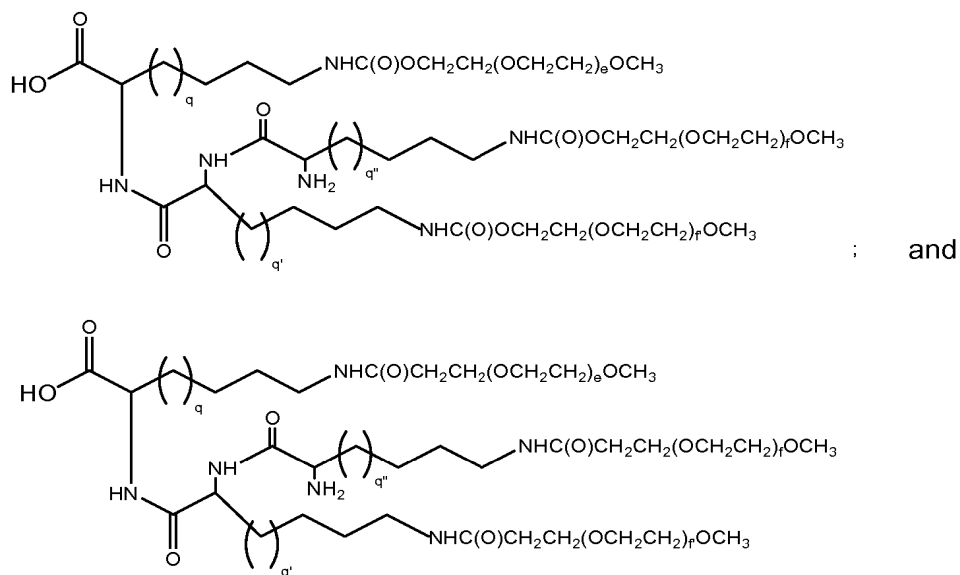
- 5 [0191] It will be appreciated by one of skill in the art that in addition to the linear PEG structures shown above, the branched polymers exemplified in the previous sections can also be attached to a branching moiety (e.g., cysteine, serine, lysine, and oligomers of lysine) in place of one or more of the linear PEG structures. In addition, those of skill will appreciate that the reliance on PEG structures set forth above is simply for clarity of illustration, the
- 10 PEG can be replaced by substantially any polymeric moiety, including, without limitation those species set forth in the definition of “polymeric moiety” found herein.



[0192] PEG of any molecular weight, e.g., 1 kD, 2 kD, 5 kD, 10 kD, 15 kD, 20 kD, 25 kD, 30 kD, 35 kD, 40 kD and 45 kD is of use in the present invention. PEG of a larger molecular weight can also be used in the present invention, including up to about 200 kD, such as at least about 180 kD, about 160 kD, about 140 kD, about 120 kD, about 100 kD, about 90 kD, about 80 kD, and about 70 kD. In certain embodiments the molecular weight of PEG is about 80 kD. In other embodiments, the molecular weight of PEG is at least about 200 kD, at least about 180 kD, at least about 160 kD, or at least about 140 kD.

[0193] Each PEG moiety of the branched polymeric modifying moiety may have a molecular weight as defined above or the total molecular weight of all PEG moieties of the polymeric modifying moiety may be as defined above. For example, in certain embodiments each PEG moiety of the branched polymeric modifying moiety may be about 80 kD or the total molecular weight of all PEG moieties of the polymeric modifying moiety may be about 80 kD. Likewise, in certain embodiments each PEG moiety of the branched polymeric modifying moiety may be about 200 kD or the total molecular weight of all PEG moieties of the polymeric modifying moiety may be about 200 kD.

[0194] Exemplary species according to this embodiment have the formulae:



in which the indices e, f and f' are independently selected integers from 1 to 2500; and the indices q, q' and q'' are independently selected integers from 1 to 20.

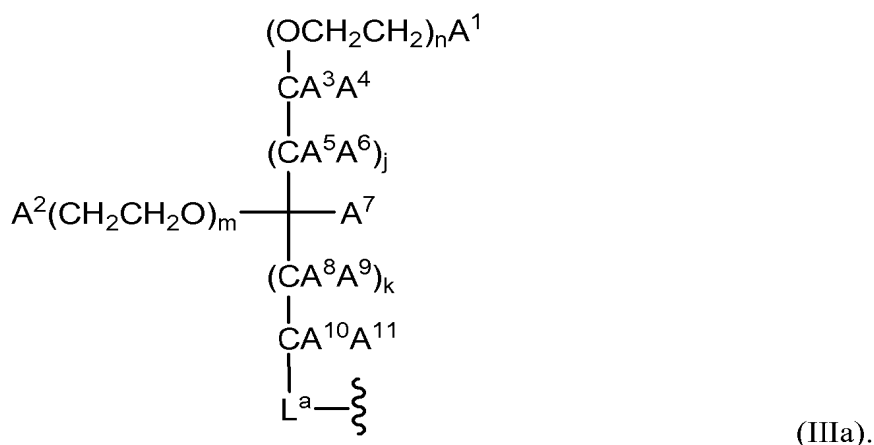
[0195] As will be apparent to those of skill, the branched polymers of use in the invention include variations on the themes set forth above. For example the di-lysine-PEG conjugate

shown above can include three polymeric subunits, the third bonded to the  $\alpha$ -amine shown as unmodified in the structure above. Similarly, the use of a tri-lysine functionalized with three or four polymeric subunits labeled with the polymeric modifying moiety in a desired manner is within the scope of the invention.

- 5 [0196] As discussed herein, the PEG of use in the conjugates of the invention can be linear or branched. An exemplary precursor of use to form the branched PEG containing peptide conjugates according to this embodiment of the invention has the formula:



- 10 Another exemplary precursor of use to form the branched PEG containing peptide conjugates according to this embodiment of the invention has the formula:



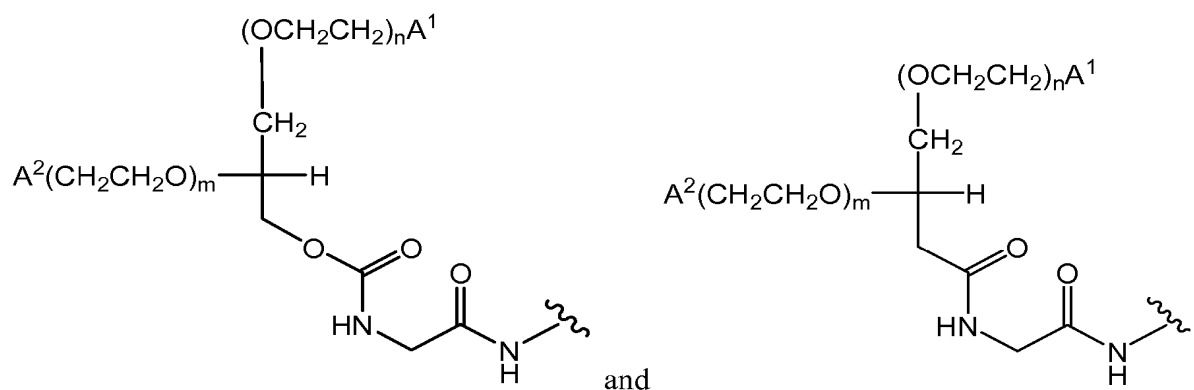
- [0197] The branched polymer species according to this formula are essentially pure water-soluble polymers.  $X^{3'}$  is a moiety that includes an ionizable (e.g., OH, COOH,  $H_2PO_4$ ,  $HSO_3$ ,  $HPO_3$ , and salts thereof, etc.) or other reactive functional group, e.g., *infra*. C is carbon.  $X^5$ ,  $R^{16}$  and  $R^{17}$  are independently selected from non-reactive groups and polymeric moieties (e.g. poly(alkylene oxide), e.g., PEG). Non-reactive groups include groups that are considered to be essentially unreactive, neutral and/ or stable at physiological pH, e.g., H, substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl and the like. An exemplary polymeric moiety includes the branched structures set forth in Formula IIIa and its exemplars. One of skill in the art will appreciate that the PEG moiety in these formulae can be replaced with other polymers. Exemplary polymers include those of the poly(alkylene oxide) family. (e.g., H, unsubstituted alkyl, unsubstituted heteroalkyl) and polymeric arms (e.g., PEG).  $X^2$  and  $X^4$  are linkage fragments that are preferably essentially non-reactive

under physiological conditions, which may be the same or different. An exemplary linker includes neither aromatic nor ester moieties. Alternatively, these linkages can include one or more moiety that is designed to degrade under physiologically relevant conditions, e.g., esters, disulfides, etc.  $X^2$  and  $X^4$  join polymeric arms  $R^{16}$  and  $R^{17}$  to C. When  $X^{3'}$  is reacted with a reactive functional group of complementary reactivity on a linker, sugar or linker-sugar cassette,  $X^{3'}$  is converted to a component of linkage fragment  $X^3$ .

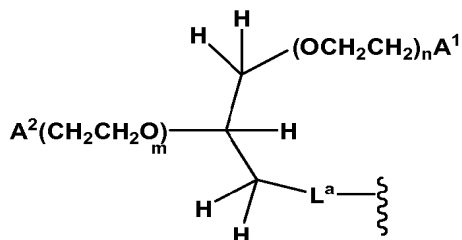
[0198] Exemplary linkage fragments for  $X^2$ ,  $X^3$  and  $X^4$  are independently selected and include S, SC(O)NH, HNC(O)S, SC(O)O, O, NH, NHC(O), (O)CNH and NHC(O)O, and OC(O)NH,  $CH_2S$ ,  $CH_2O$ ,  $CH_2CH_2O$ ,  $CH_2CH_2S$ ,  $(CH_2)_oO$ ,  $(CH_2)_oS$  or  $(CH_2)_oY'$ -PEG wherein,  $Y'$  is S, NH, NHC(O), C(O)NH, NHC(O)O, OC(O)NH, or O and o is an integer from 1 to 50. In an exemplary embodiment, the linkage fragments  $X^2$  and  $X^4$  are different linkage fragments.

[0199] In an exemplary embodiment, the precursor (Formula III), or an activated derivative thereof, is reacted with, and thereby bound to a sugar, an activated sugar or a sugar nucleotide through a reaction between  $X^{3'}$  and a group of complementary reactivity on the sugar moiety, e.g., an amine. Alternatively,  $X^{3'}$  reacts with a reactive functional group on a precursor to linker, L. One or more of  $R^2$ ,  $R^3$ ,  $R^4$ ,  $R^5$ ,  $R^6$  or  $R^{6'}$  of Formulae I, Ia, II or IIa can include the branched polymeric modifying moiety, or this moiety bound through L.

[0200] In an exemplary embodiment, the polymeric modifying group has a structure including a moiety according to the following formulae:

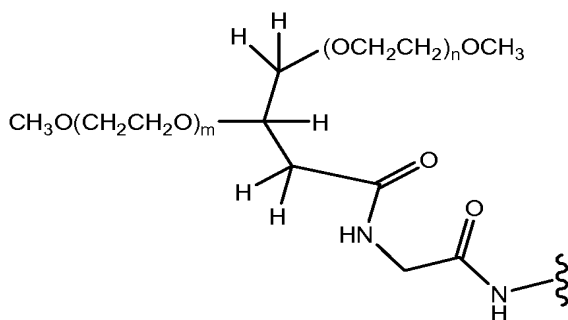
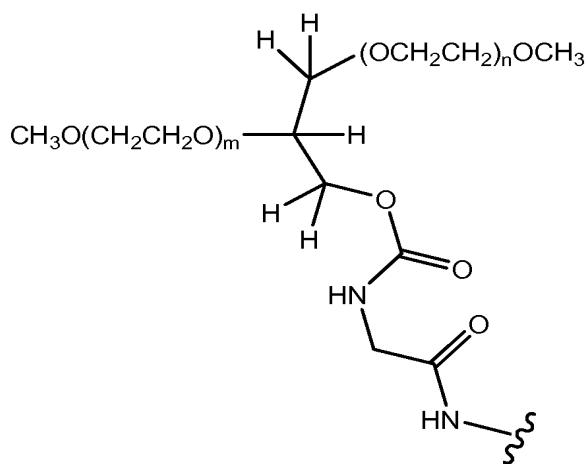


[0201] In another exemplary embodiment according to the formula above, the branched polymer has a structure according to the following formula:



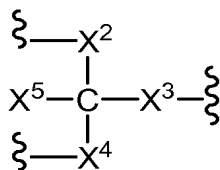
In an exemplary embodiment,  $A^1$  and  $A^2$  are each selected from  $-OH$  and  $-OCH_3$ .

**[0202]** Exemplary polymeric modifying groups according to this embodiment include the moiety:

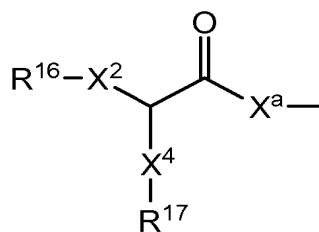


and

**[0203]** In an exemplary embodiment, the moiety:



is the linker arm,  $L$ . In this embodiment, an exemplary linker is derived from a natural or unnatural amino acid, amino acid analogue or amino acid mimetic, or a small peptide formed from one or more such species. For example, certain branched polymers found in the compounds of the invention have the formula:

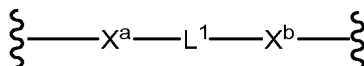


(IV).

**[0204]**  $X^a$  is a linkage fragment that is formed by the reaction of a reactive functional group, e.g.,  $X^{3'}$ , on a precursor of the branched polymeric modifying moiety and a reactive

functional group on the sugar moiety, or a precursor to a linker. For example, when  $X^{3'}$  is a carboxylic acid, it can be activated and bound directly to an amine group pendent from an amino-saccharide (e.g., Sia, GalNH<sub>2</sub>, GlcNH<sub>2</sub>, ManNH<sub>2</sub>, etc.), forming a  $X^a$  that is an amide. Additional exemplary reactive functional groups and activated precursors are described hereinbelow. The index c represents an integer from 1 to 10. The other symbols have the same identity as those discussed above.

[0205] In another exemplary embodiment,  $X^a$  is a linking moiety formed with another linker:

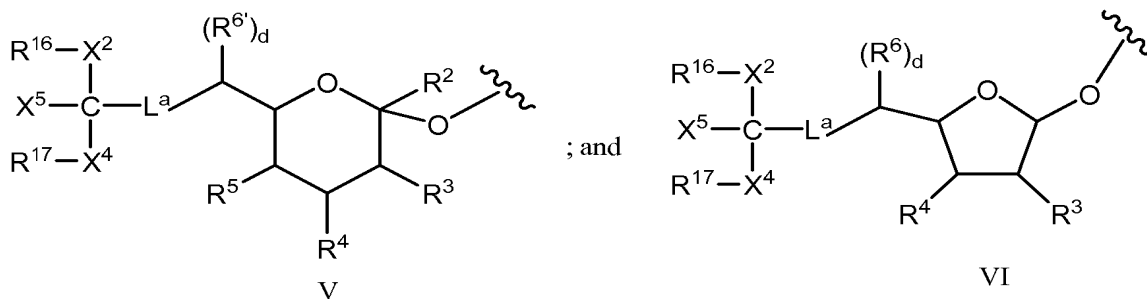


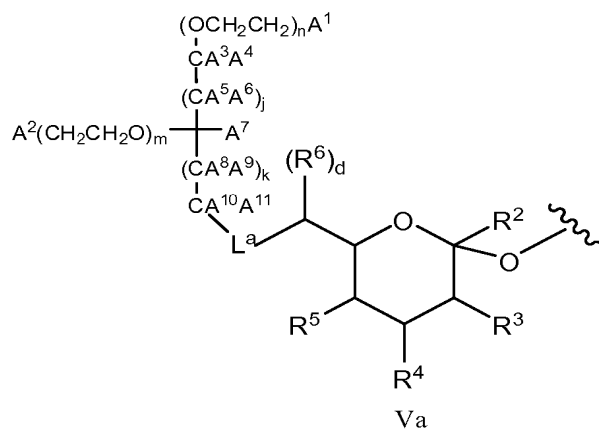
in which  $X^b$  is a second linkage fragment and is independently selected from those groups set forth for  $X^a$ , and, similar to L,  $L^1$  is a bond, substituted or unsubstituted alkyl or substituted or unsubstituted heteroalkyl.

[0206] Exemplary species for  $X^a$  and  $X^b$  include S, SC(O)NH, HNC(O)S, SC(O)O, O, NH, NHC(O), C(O)NH and NHC(O)O, and OC(O)NH.

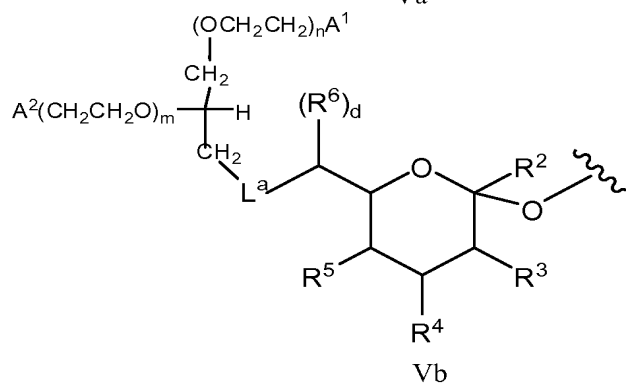
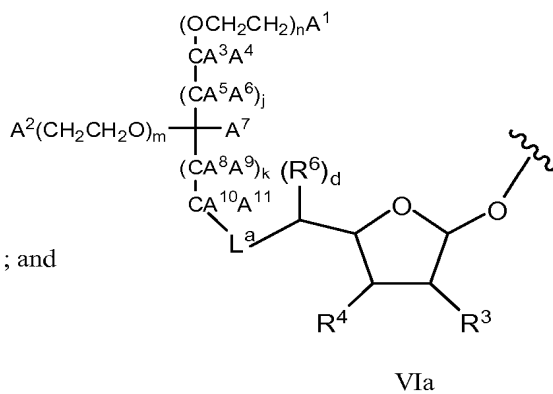
[0207] In another exemplary embodiment,  $X^4$  is a peptide bond to  $R^{17}$ , which is an amino acid, di-peptide (e.g., Lys-Lys) or tri-peptide (e.g., Lys-Lys-Lys) in which the alpha-amine moiety(ies) and/or side chain heteroatom(s) are modified with a polymeric modifying moiety.

[0208] In a further exemplary embodiment, the peptide conjugates of the invention include a moiety, e.g., an  $R^{15}$  moiety that has a formula that is selected from:

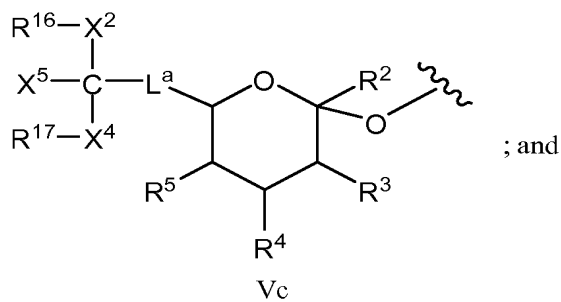
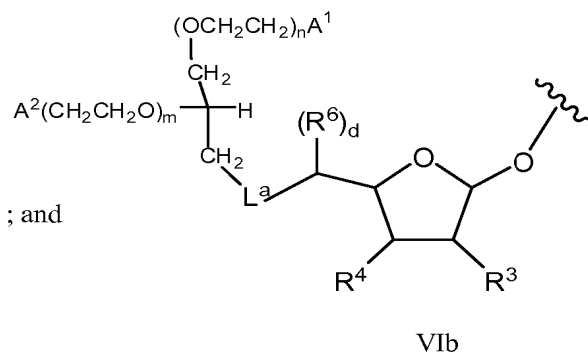




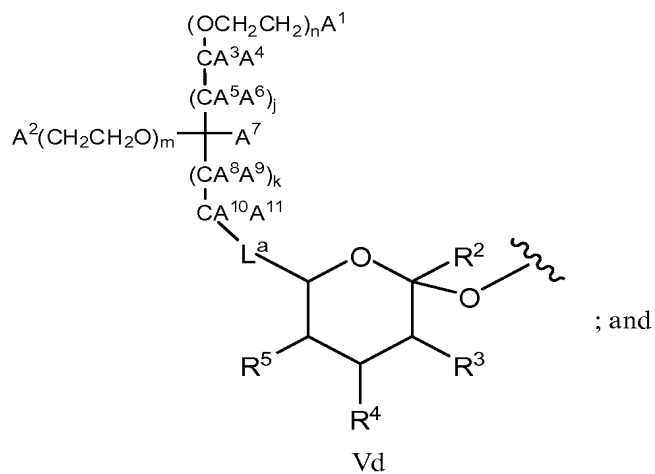
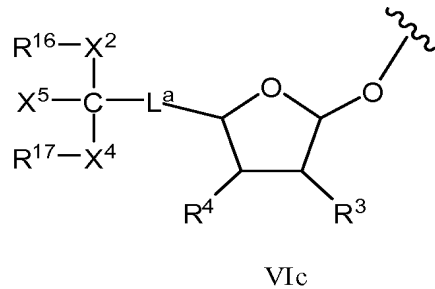
; and



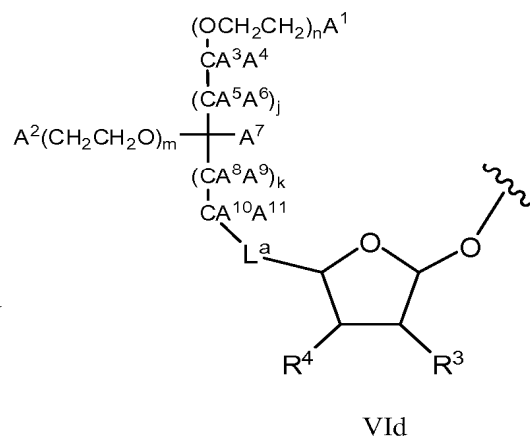
; and

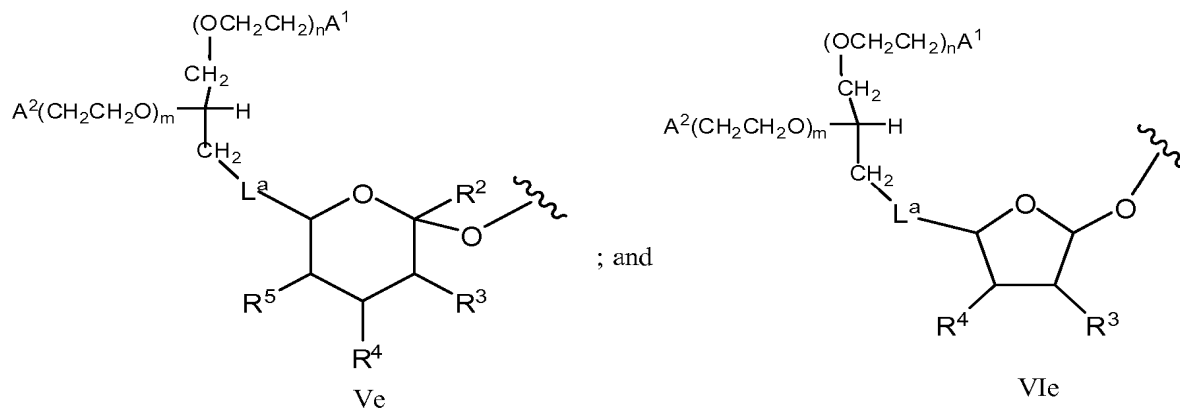


; and



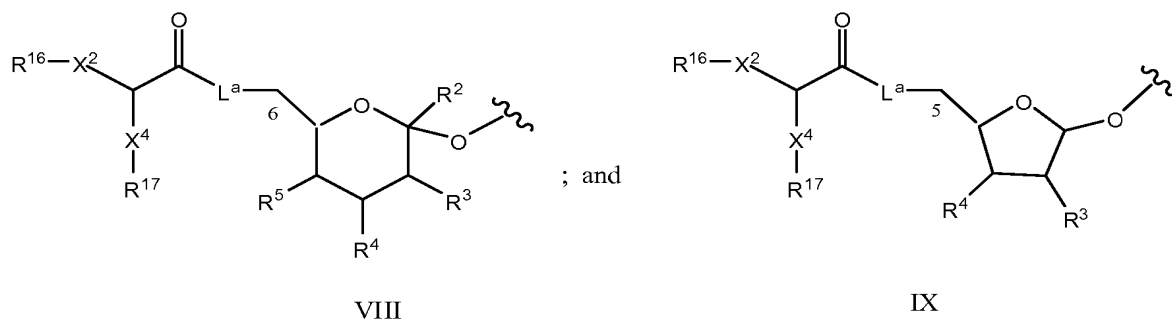
; and





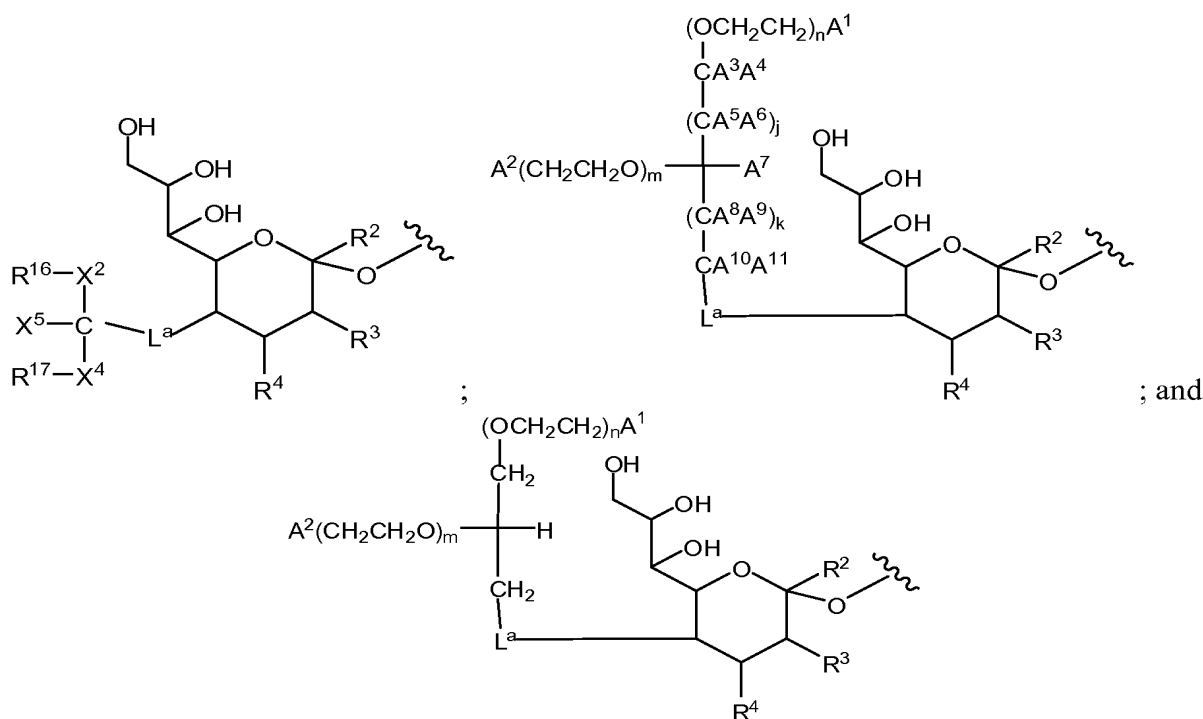
in which the identity of the radicals represented by the various symbols is the same as that discussed hereinabove.  $L^a$  is a bond or a linker as discussed above for  $L$  and  $L^1$ , e.g., substituted or unsubstituted alkyl or substituted or unsubstituted heteroalkyl moiety. In an exemplary embodiment,  $L^a$  is a moiety of the side chain of sialic acid that is functionalized with the polymeric modifying moiety as shown. Exemplary  $L^a$  moieties include substituted or unsubstituted alkyl chains that include one or more OH or  $NH_2$ .

[0209] In yet another exemplary embodiment, the invention provides peptide conjugates having a moiety, e.g., an  $R^{15}$  moiety with formula:



The identity of the radicals represented by the various symbols is the same as that discussed hereinabove. As those of skill will appreciate, the linker arm in Formulae VIII and IX is equally applicable to other modified sugars set forth herein. In exemplary embodiment, the species of Formulae VIII and IX are the  $R^{15}$  moieties attached to the glycan structures set forth herein.

[0210] In yet another exemplary embodiment, the peptide conjugate includes a  $R^{15}$  moiety with a formula which is a member selected from:

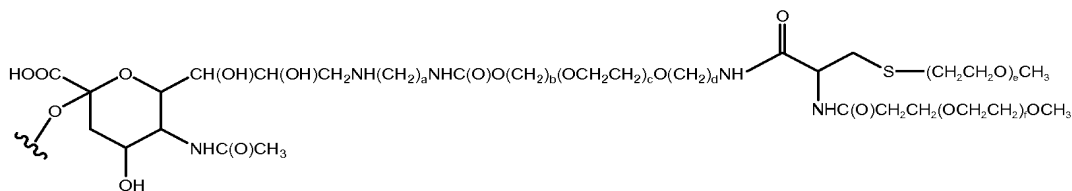
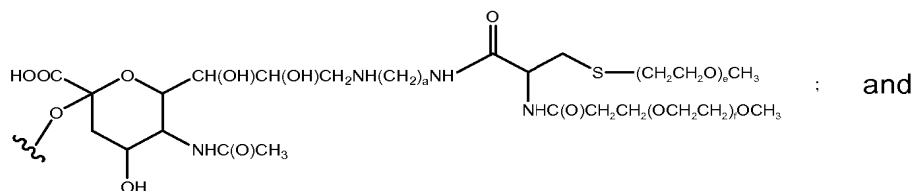
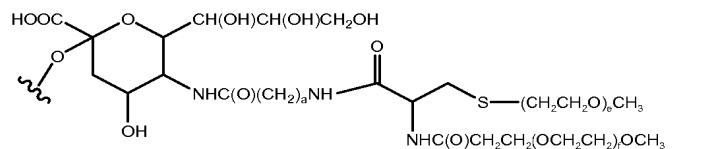
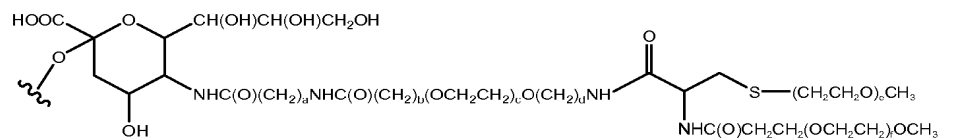


in which the identities of the radicals are as discussed above. An exemplary species for  $L^a$  is  $-(CH_2)_jC(O)NH(CH_2)_hC(O)NH-$ , in which the indices  $h$  and  $j$  are independently selected integers from 0 to 10. A further exemplary species is  $-C(O)NH-$ . The indices  $m$  and  $n$  are integers independently selected from 0 to 5000.  $A^1$ ,  $A^2$ ,  $A^3$ ,  $A^4$ ,  $A^5$ ,  $A^6$ ,  $A^7$ ,  $A^8$ ,  $A^9$ ,  $A^{10}$  and  $A^{11}$  are members independently selected from H, substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl, substituted or unsubstituted cycloalkyl, substituted or unsubstituted heterocycloalkyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl,  $-NA^{12}A^{13}$ ,  $-OA^{12}$  and  $-SiA^{12}A^{13}$ .  $A^{12}$  and  $A^{13}$  are members independently selected from substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl, substituted or unsubstituted cycloalkyl, substituted or unsubstituted heterocycloalkyl, substituted or unsubstituted aryl, and substituted or unsubstituted heteroaryl.

**[0211]** The embodiments of the invention set forth above are further exemplified by reference to species in which the polymer is a water-soluble polymer, particularly poly(ethylene glycol) ("PEG"), e.g., methoxy-poly(ethylene glycol). Those of skill will appreciate that the focus in the sections that follow is for clarity of illustration and the various motifs set forth using PEG as an exemplary polymer are equally applicable to species in which a polymer other than PEG is utilized.

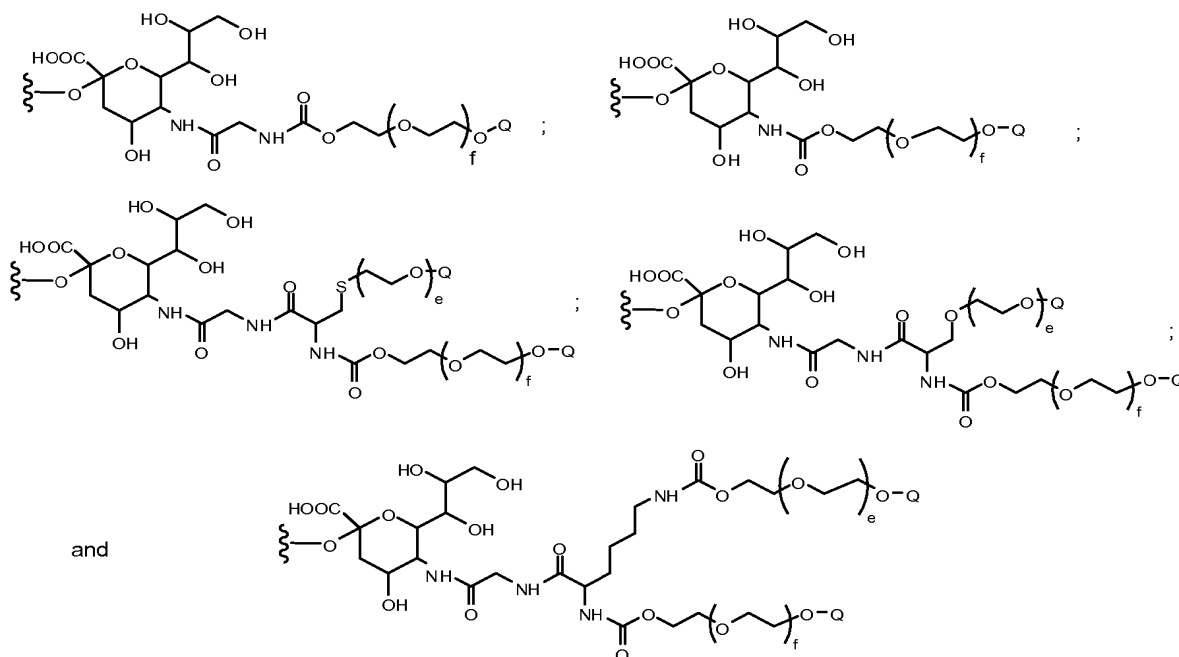


[0212] In an exemplary embodiment, the R<sup>15</sup> moiety has a formula that is a member selected from the group:



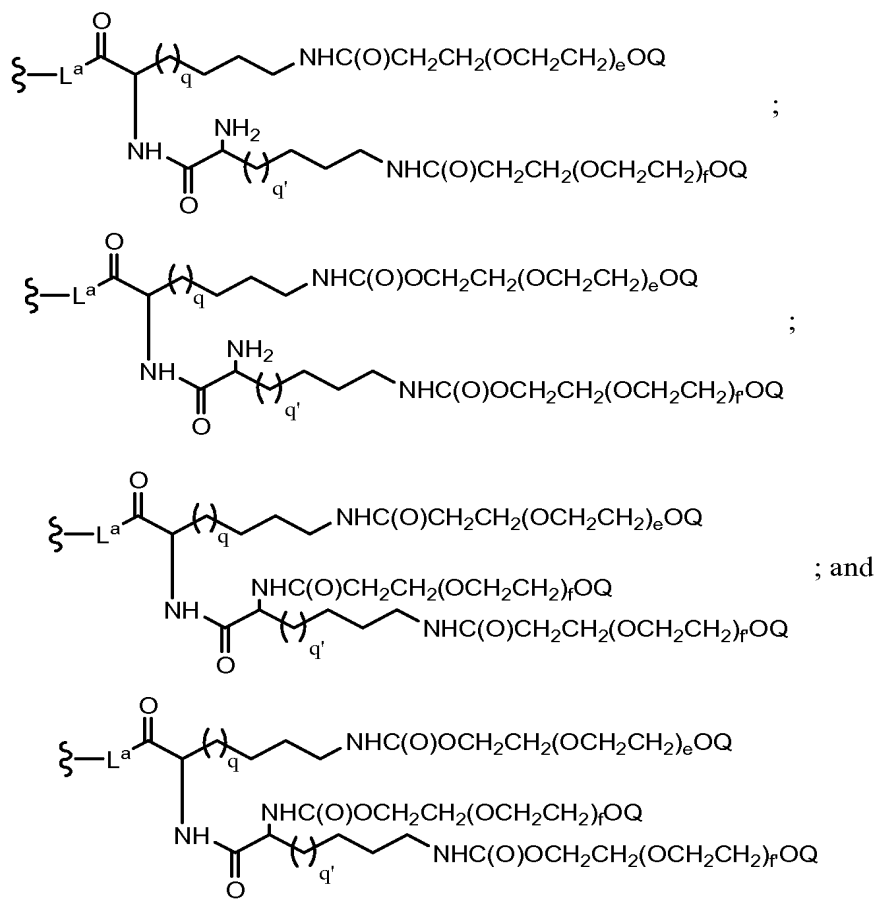
In each of the structures above, the linker fragment  $-\text{NH}(\text{CH}_2)_a-$  can be present or absent.

5 [0213] In other exemplary embodiments, the peptide conjugate includes an R<sup>15</sup> moiety selected from the group:

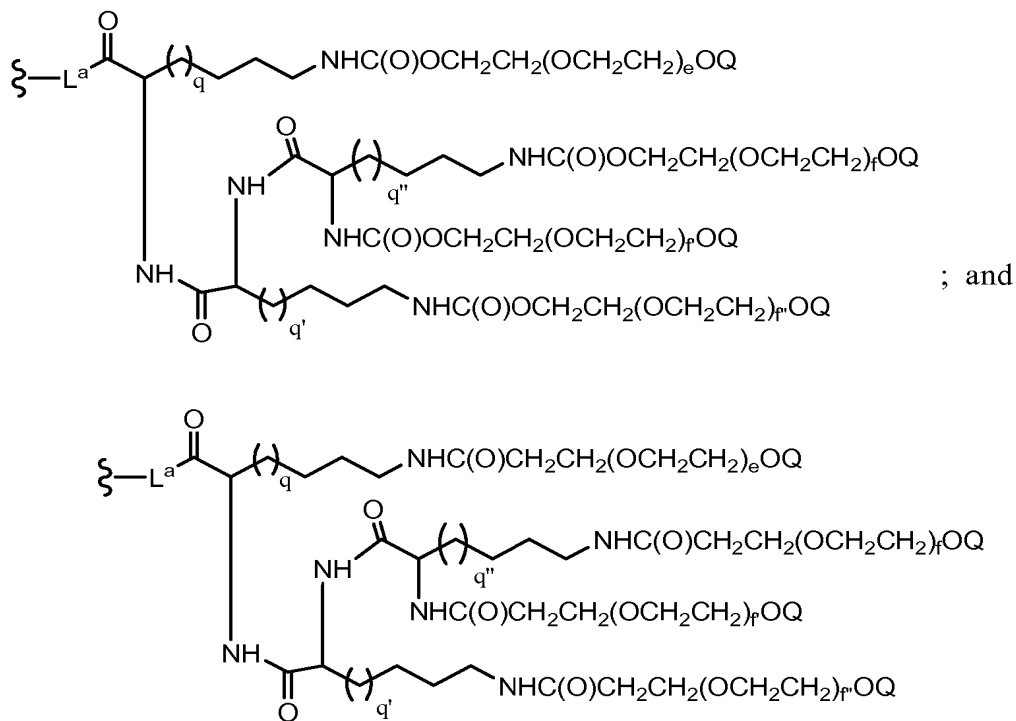


[0214] In each of the formulae above, the indices  $e$  and  $f$  are independently selected from the integers from 1 to 2500. In further exemplary embodiments,  $e$  and  $f$  are selected to provide a PEG moiety that is about 1 kD, 2 kD, 5 kD, 10 kD, 15 kD, 20 kD, 25 kD, 30 kD, 35 kD, 40 kD and 45 kD. PEG of a larger molecular weight can also be used in the present invention, including up to about 200 kD, such as at least about 180 kD, about 160 kD, about 140 kD, about 120 kD, about 100 kD, about 90 kD, about 80 kD, and about 70 kD. In certain embodiments the molecular weight of PEG is about 80 kD. In other embodiments, the molecular weight of PEG is at least about 200 kD, at least about 180 kD, at least about 160 kD, or at least about 140 kD. The symbol  $Q$  represents substituted or unsubstituted alkyl (e.g.,  $C_1$ - $C_6$  alkyl, e.g., methyl), substituted or unsubstituted heteroalkyl or H.

[0215] Other branched polymers have structures based on di-lysine (Lys-Lys) peptides, e.g.:

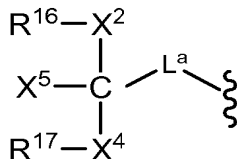


and tri-lysine peptides (Lys-Lys-Lys), e.g.:

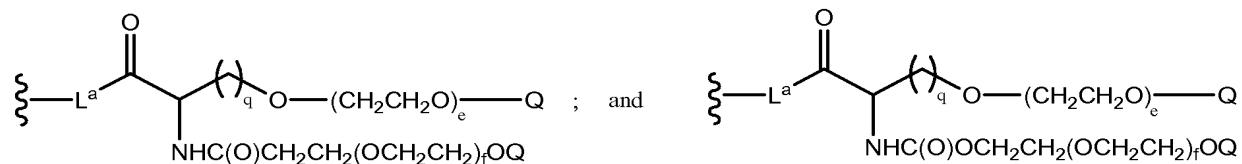
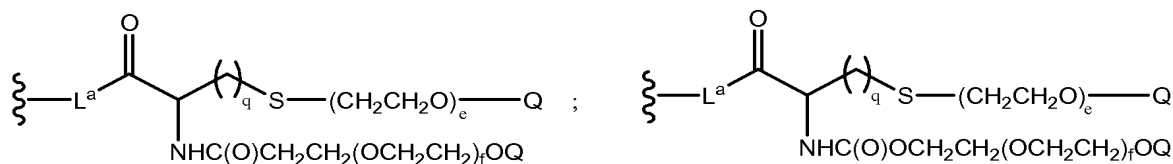


In each of the figures above, the indices e, f, f' and f'' represent integers independently selected from 1 to 2500. The indices q, q' and q'' represent integers independently selected from 1 to 20. It will be appreciated by one of skill in the art that in addition to the linear PEG structures shown above, the branched polymers exemplified in the previous sections can also be attached to a branching moiety (e.g., lysine, and oligomers of lysine) in place of one or more of the linear PEG structures.

[0216] In another exemplary embodiment, the modifying group:

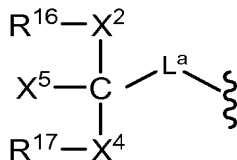


has a formula that is a member selected from:

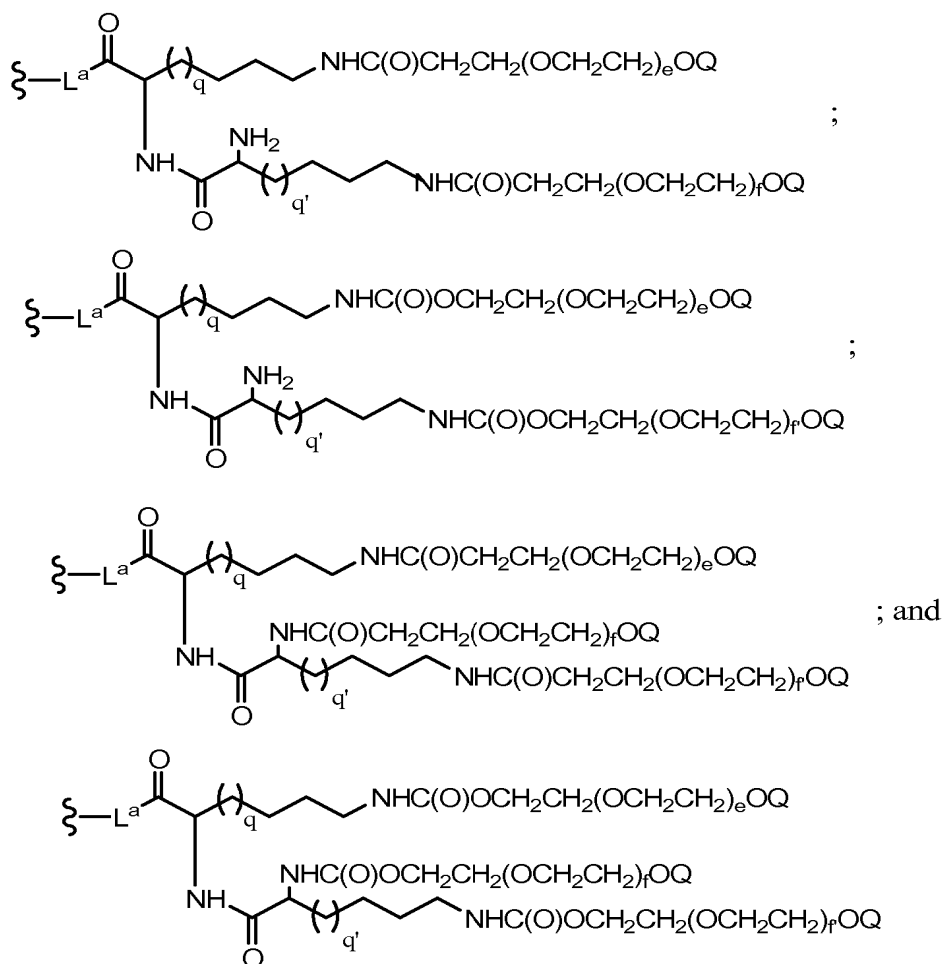


wherein Q is a member selected from H and substituted or unsubstituted C<sub>1</sub>-C<sub>6</sub> alkyl. The indices e and f are integers independently selected from 1 to 2500, and the index q is an integer selected from 0 to 20.

[0217] In another exemplary embodiment, the modifying group:

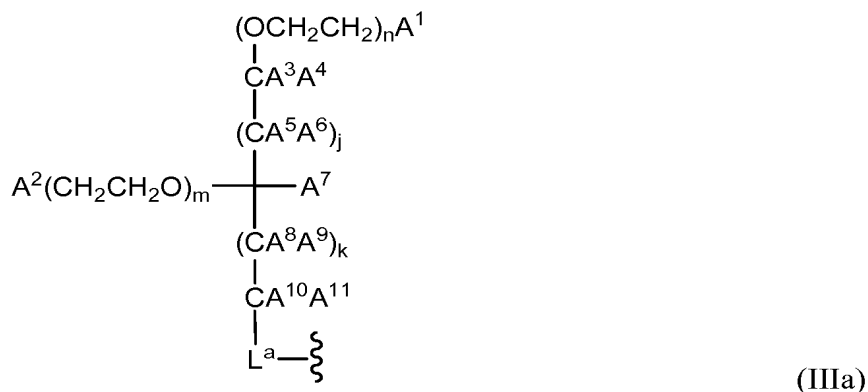


has a formula that is a member selected from:



wherein Q is a member selected from H and substituted or unsubstituted C<sub>1</sub>-C<sub>6</sub> alkyl. The indices e, f and f' are integers independently selected from 1 to 2500, and q and q' are integers independently selected from 1 to 20.

- 5 [0218] In another exemplary embodiment, the branched polymer has a structure including a moiety according to the following formula:

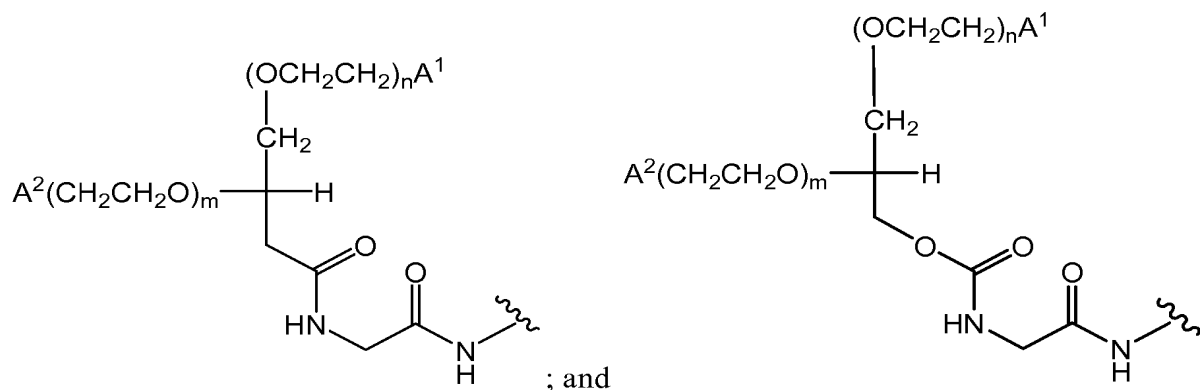


in which the indices m and n are integers independently selected from 0 to 5000. A<sup>1</sup>, A<sup>2</sup>, A<sup>3</sup>, A<sup>4</sup>, A<sup>5</sup>, A<sup>6</sup>, A<sup>7</sup>, A<sup>8</sup>, A<sup>9</sup>, A<sup>10</sup> and A<sup>11</sup> are members independently selected from H, substituted

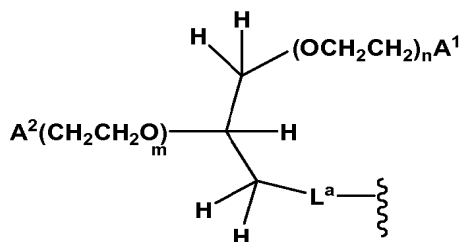
or unsubstituted alkyl, substituted or unsubstituted heteroalkyl, substituted or unsubstituted cycloalkyl, substituted or unsubstituted heterocycloalkyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl,  $-\text{NA}^{12}\text{A}^{13}$ ,  $-\text{OA}^{12}$  and  $-\text{SiA}^{12}\text{A}^{13}$ .  $\text{A}^{12}$  and  $\text{A}^{13}$  are members independently selected from substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl, substituted or unsubstituted cycloalkyl, substituted or unsubstituted heterocycloalkyl, substituted or unsubstituted aryl, and substituted or unsubstituted heteroaryl.

[0219] Formula IIIa is a subset of Formula III. The structures described by Formula IIIa are also encompassed by Formula III.

- 10 [0220] In an exemplary embodiment, the polymeric modifying group has a structure including a moiety according to the following formulae:

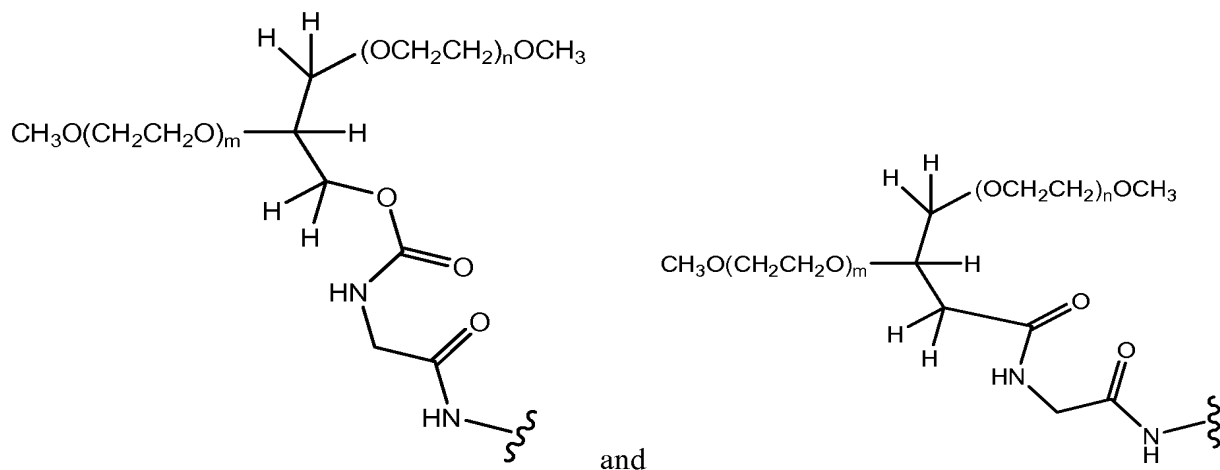


[0221] In another exemplary embodiment according to the formula above, the branched polymer has a structure including a moiety according to the following formula:



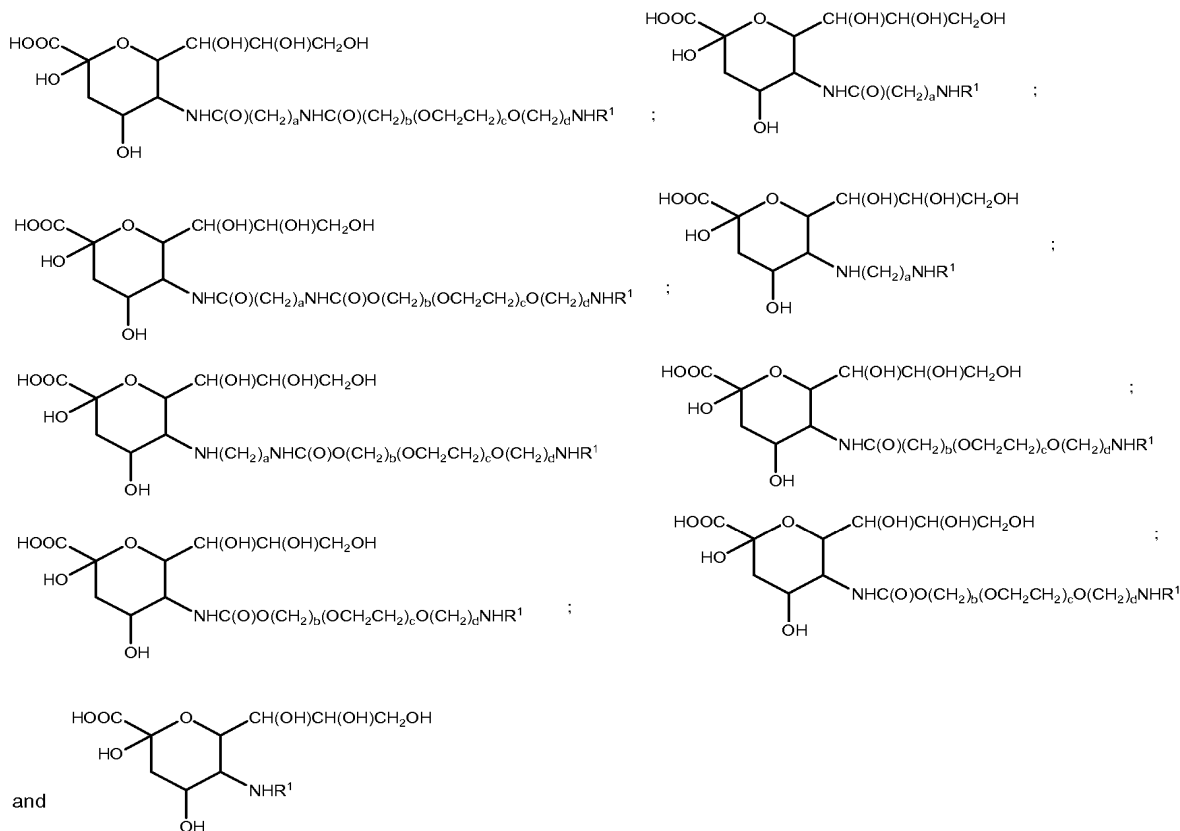
In an exemplary embodiment,  $\text{A}^1$  and  $\text{A}^2$  are members independently selected from  $-\text{OH}$  and  $-\text{OCH}_3$ .

[0222] Exemplary polymeric modifying groups according to this embodiment include the moiety:



wherein the variables are as described above.

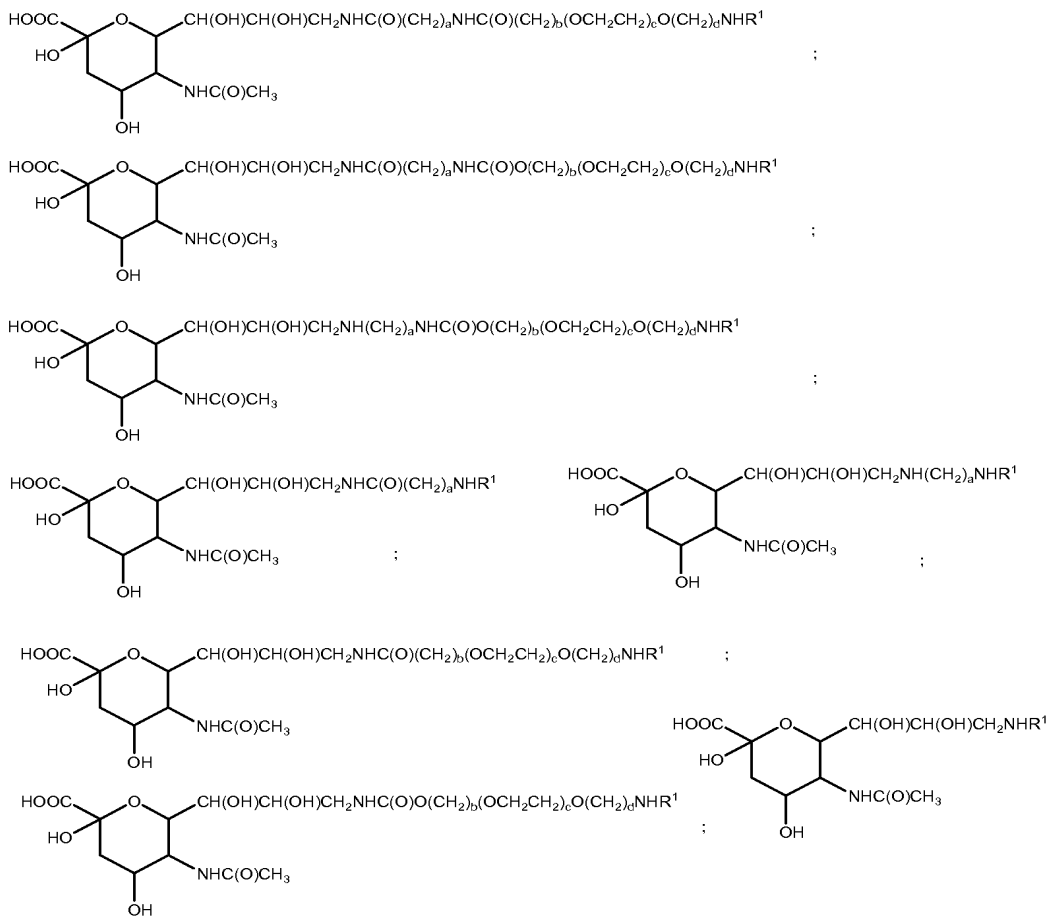
5 [0223] In an illustrative embodiment, the modified sugar is sialic acid and selected modified sugar compounds of use in the invention have the formulae:



The indices a, b and d are integers from 0 to 20. The index c is an integer from 1 to 2500.

The structures set forth above can be components of  $R^{15}$ .

[0224] In another illustrative embodiment, a primary hydroxyl moiety of the sugar is functionalized with the modifying group. For example, the 9-hydroxyl of sialic acid can be converted to the corresponding amine and functionalized to provide a compound according to the invention. Formulae according to this embodiment include:

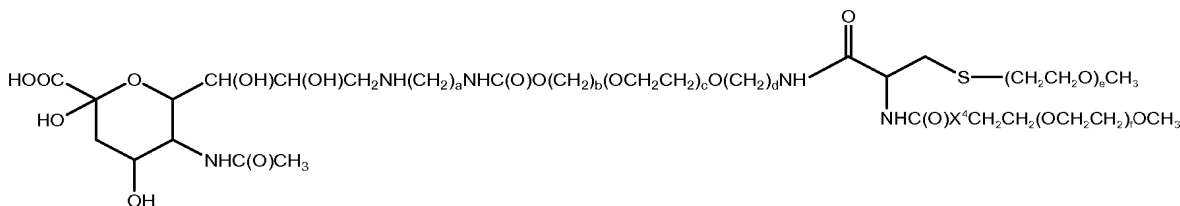
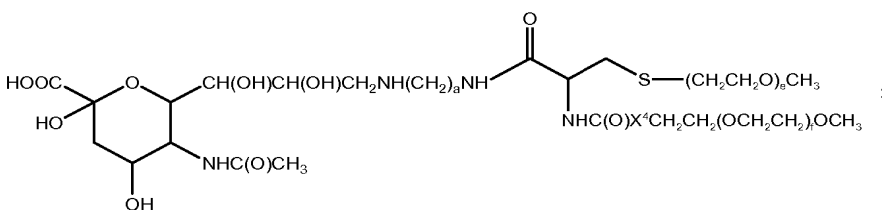
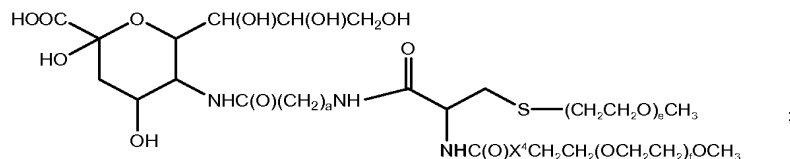
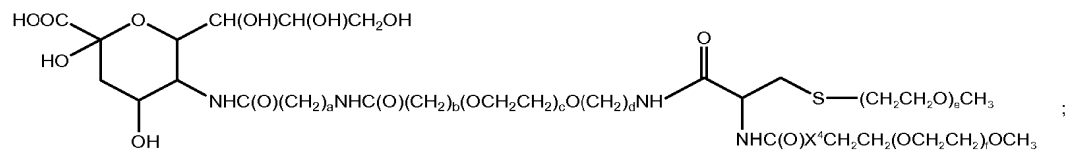


The structures set forth above can be components of  $R^{15}$ .

[0225] Although the present invention is exemplified in the preceding sections by reference to PEG, as those of skill will appreciate, an array of polymeric modifying moieties is of use in the compounds and methods set forth herein.

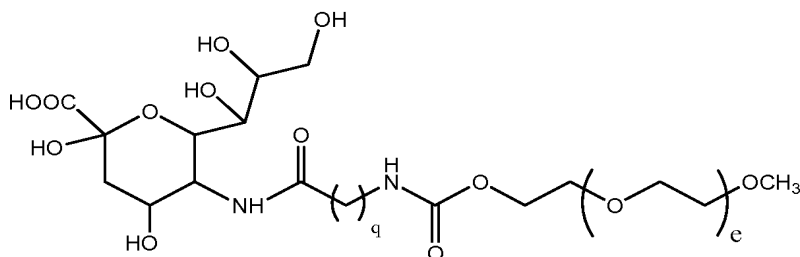
[0226] In selected embodiments,  $R^1$  or  $L-R^1$  is a branched PEG, for example, one of the species set forth above. In an exemplary embodiment, the branched PEG structure is based on a cysteine peptide. Illustrative modified sugars according to this embodiment include:





in which  $X^4$  is a bond or O. In each of the structures above, the alkylamine linker  $-(CH_2)_aNH-$  can be present or absent. The structures set forth above can be components of  $R^{15}/R^{15'}$ .

- 5 [0227] As discussed herein, the polymer-modified sialic acids of use in the invention may also be linear structures. Thus, the invention provides for conjugates that include a sialic acid moiety derived from a structure such as:

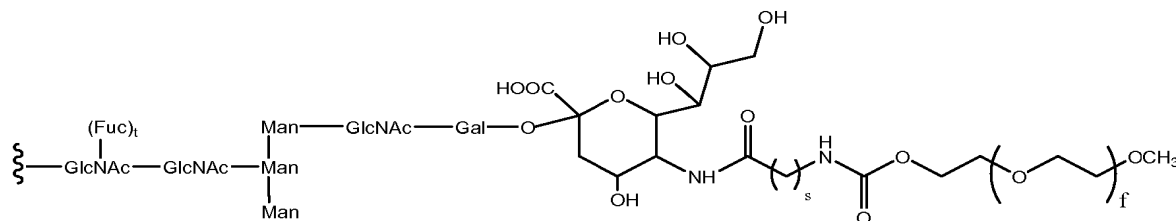


in which the indices  $q$  and  $e$  are as discussed above.

- 10 [0228] Exemplary modified sugars are modified with water-soluble or water-insoluble polymers. Examples of useful polymer are further exemplified below.

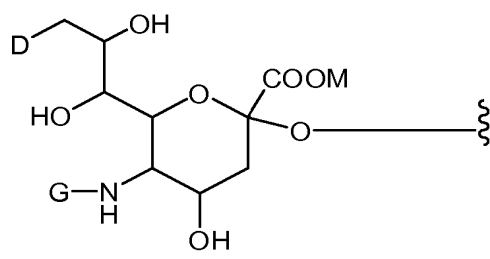
[0229] In another exemplary embodiment, the peptide is derived from insect cells, remodeled by adding GlcNAc and Gal to the mannose core and glycopegylated using a sialic

acid bearing a linear PEG moiety, affording a peptide that comprises at least one moiety having the formula:



in which the index  $t$  is an integer from 0 to 1; the index  $s$  represents an integer from 1 to 10;  
5 and the index  $f$  represents an integer from 1 to 2500.

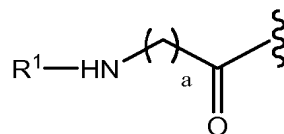
**[0230]** In one embodiment, the present invention provides a peptide conjugate comprising the following glycosyl linking group:



;

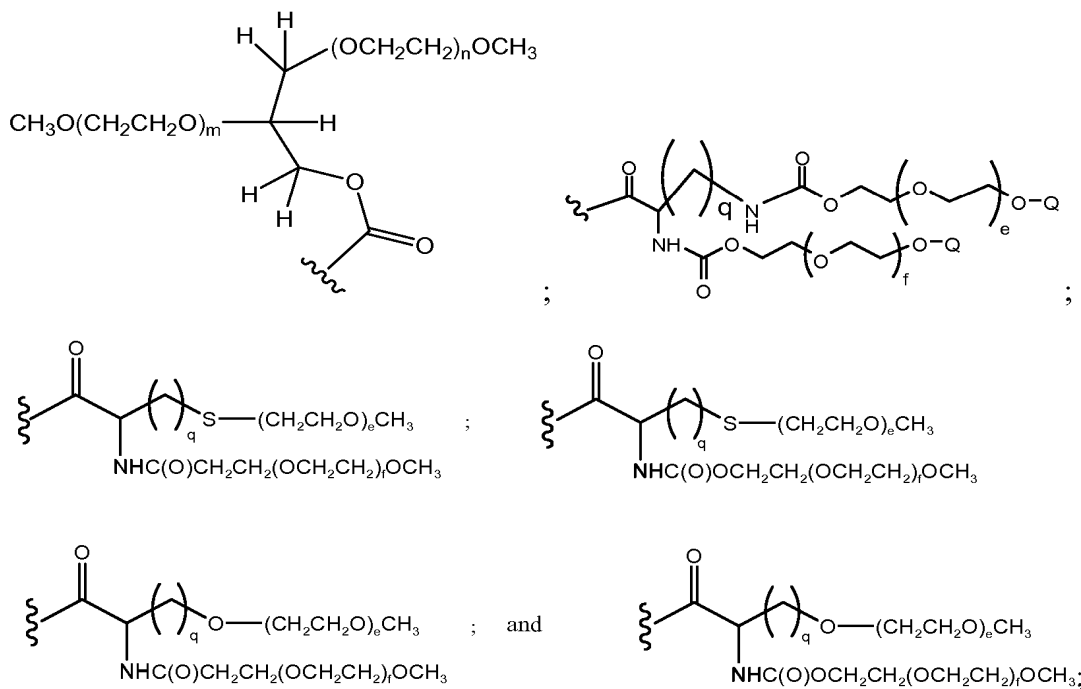
$D$  is a member selected from  $-OH$  and  $R^1-L-NH-$ ;  $G$  is a member selected from  $R^1-L-$  and  
10  $-C(O)(C_1-C_6)alkyl-R^1$ ;  $R^1$  is a moiety comprising a member selected from a straight-chain poly(ethylene glycol) residue and branched poly(ethylene glycol) residue; and  
 $M$  is a member selected from  $H$ , a salt counterion and a single negative charge;  $L$  is a linker which is a member selected from a bond, substituted or unsubstituted alkyl and substituted or unsubstituted heteroalkyl. In an exemplary embodiment, when  $D$  is  $OH$ ,  $G$  is  $R^1-L-$ . In  
15 another exemplary embodiment, when  $G$  is  $-C(O)(C_1-C_6)alkyl$ ,  $D$  is  $R^1-L-NH-$ .

**[0231]** In an exemplary embodiment,  $L-R^1$  has the formula:



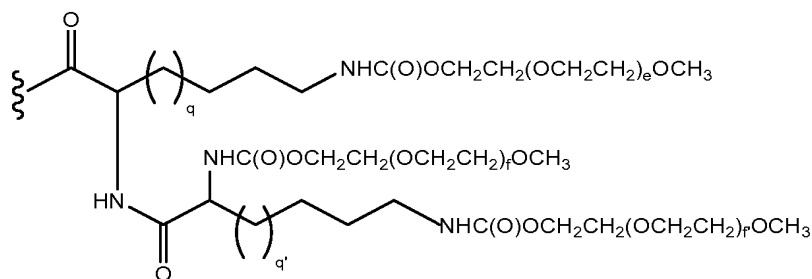
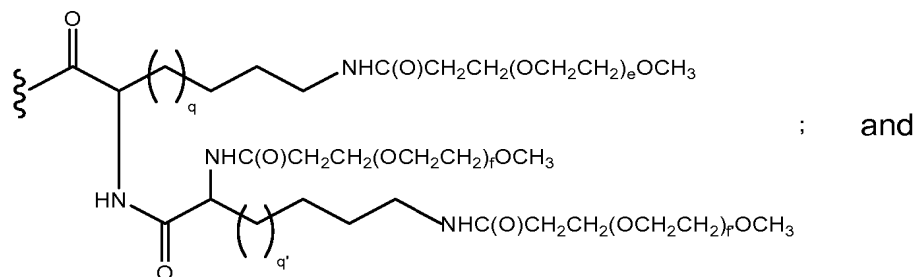
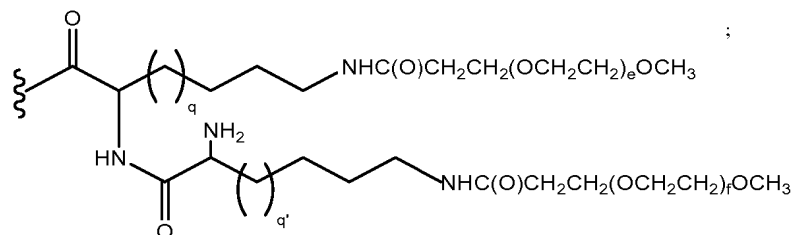
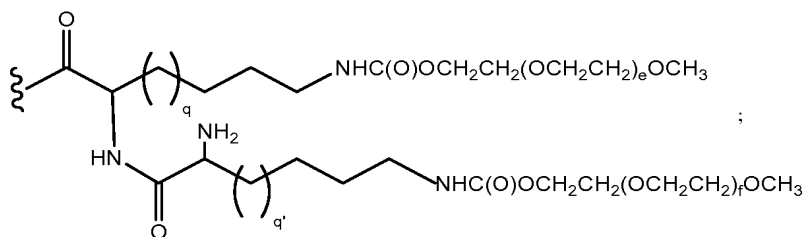
wherein  $a$  is an integer selected from 0 to 20.

**[0232]** In an exemplary embodiment,  $R^1$  has a structure that includes a moiety selected  
20 from:



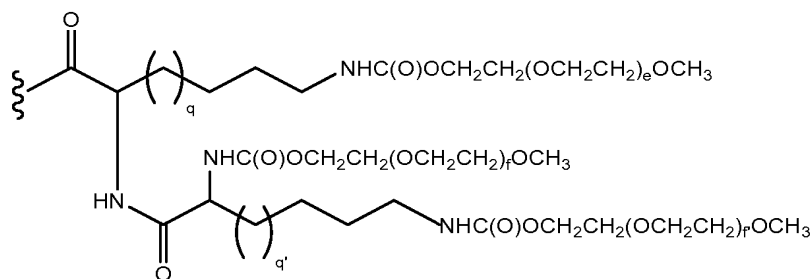
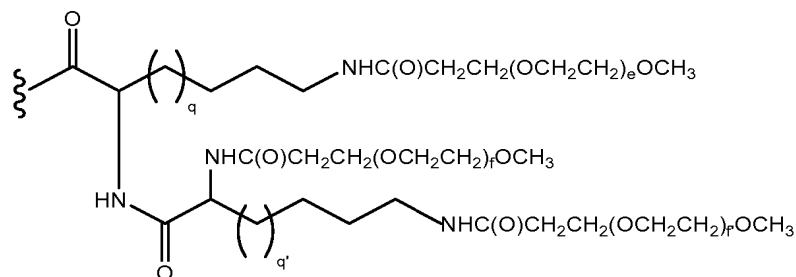
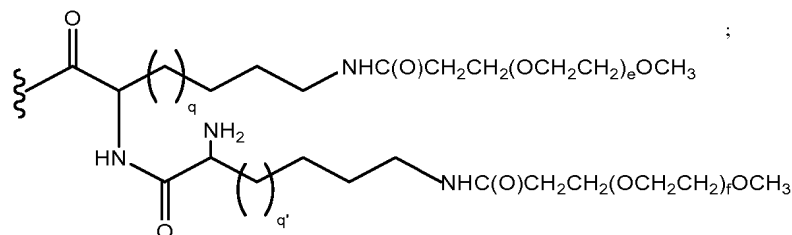
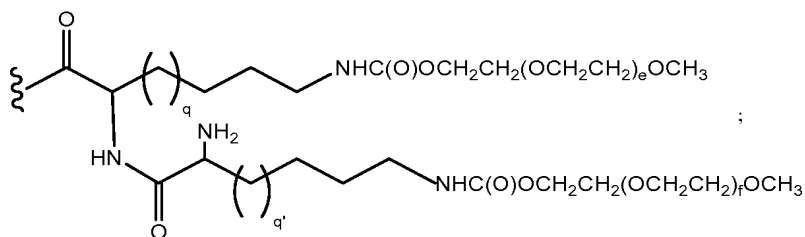
wherein e, f, m and n are integers independently selected from 1 to 2500; and q is an integer selected from 0 to 20.

- 5 [0233] In an exemplary embodiment,  $\text{R}^1$  has a structure that is a member selected from:



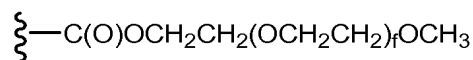
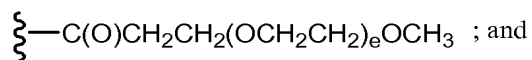
wherein e, f and f' are integers independently selected from 1 to 2500; and q and q' are integers independently selected from 1 to 20.

**[0234]** In another exemplary embodiment,  $R^1$  has a structure that is a member selected from:



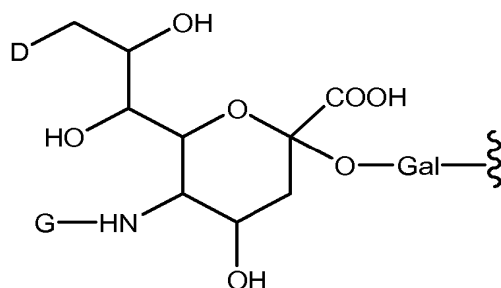
wherein e, f and f' are integers independently selected from 1 to 2500; and q and q' are integers independently selected from 1 to 20.

[0235] In another exemplary embodiment, R<sup>1</sup> has a structure that is a member selected from:



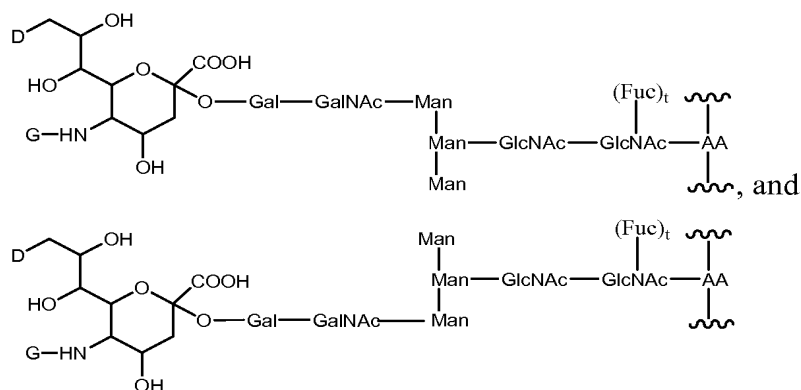
wherein e and f are integers independently selected from 1 to 2500.

[0236] In another exemplary embodiment, the glycosyl linker has the formula:



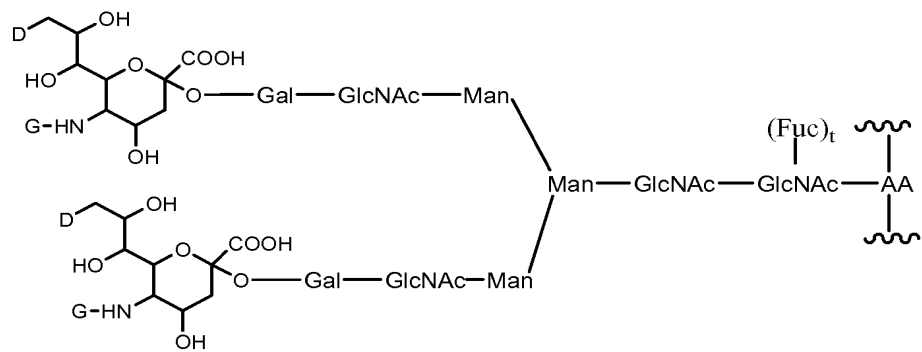
wherein the variables are as described above.

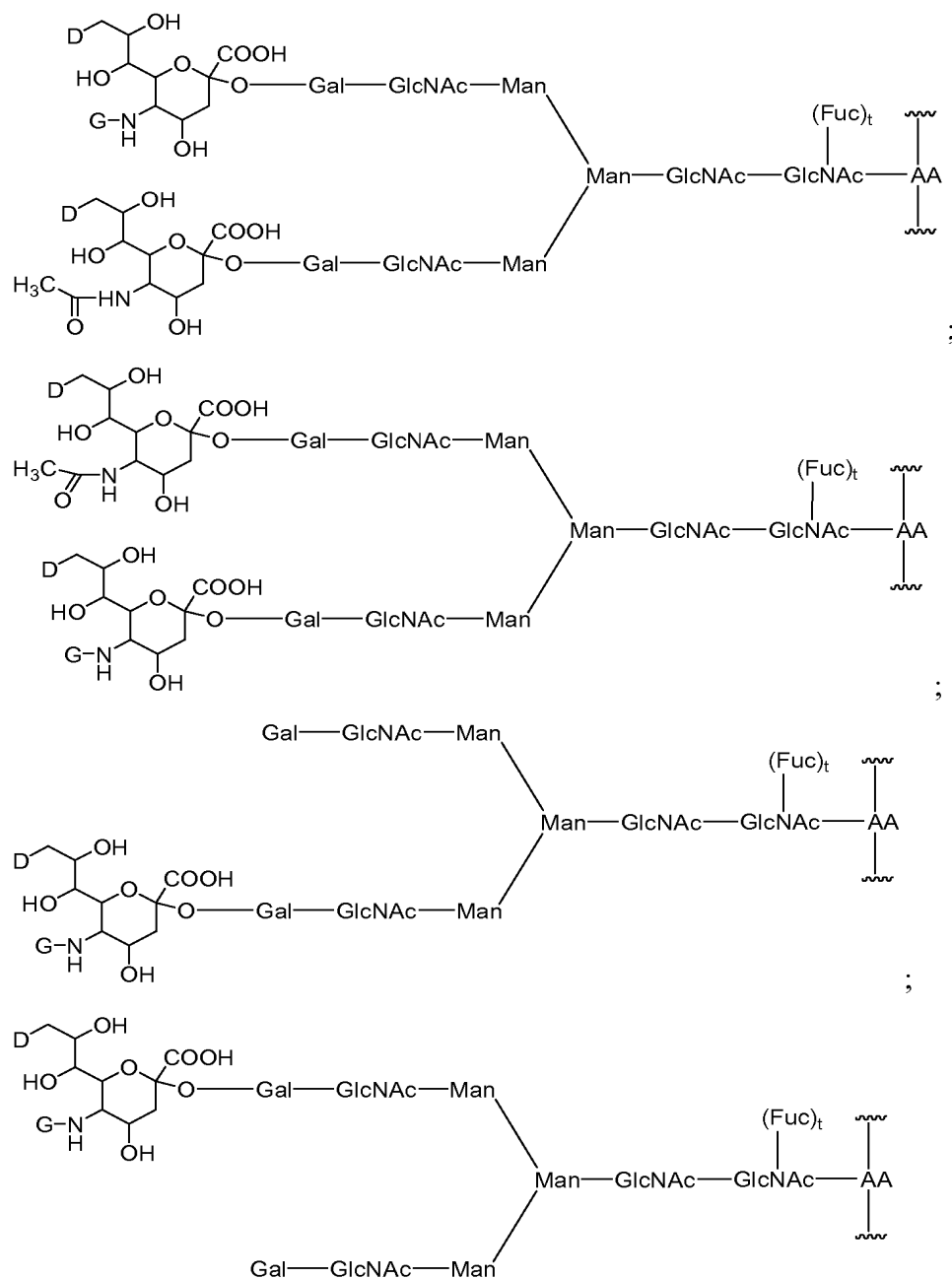
[0237] In another exemplary embodiment, the peptide conjugate comprises at least one of said glycosyl linker according to a formula selected from:



wherein D and G are as described above, AA is an amino acid residue of said peptide conjugate and t is an integer selected from 0 and 1.

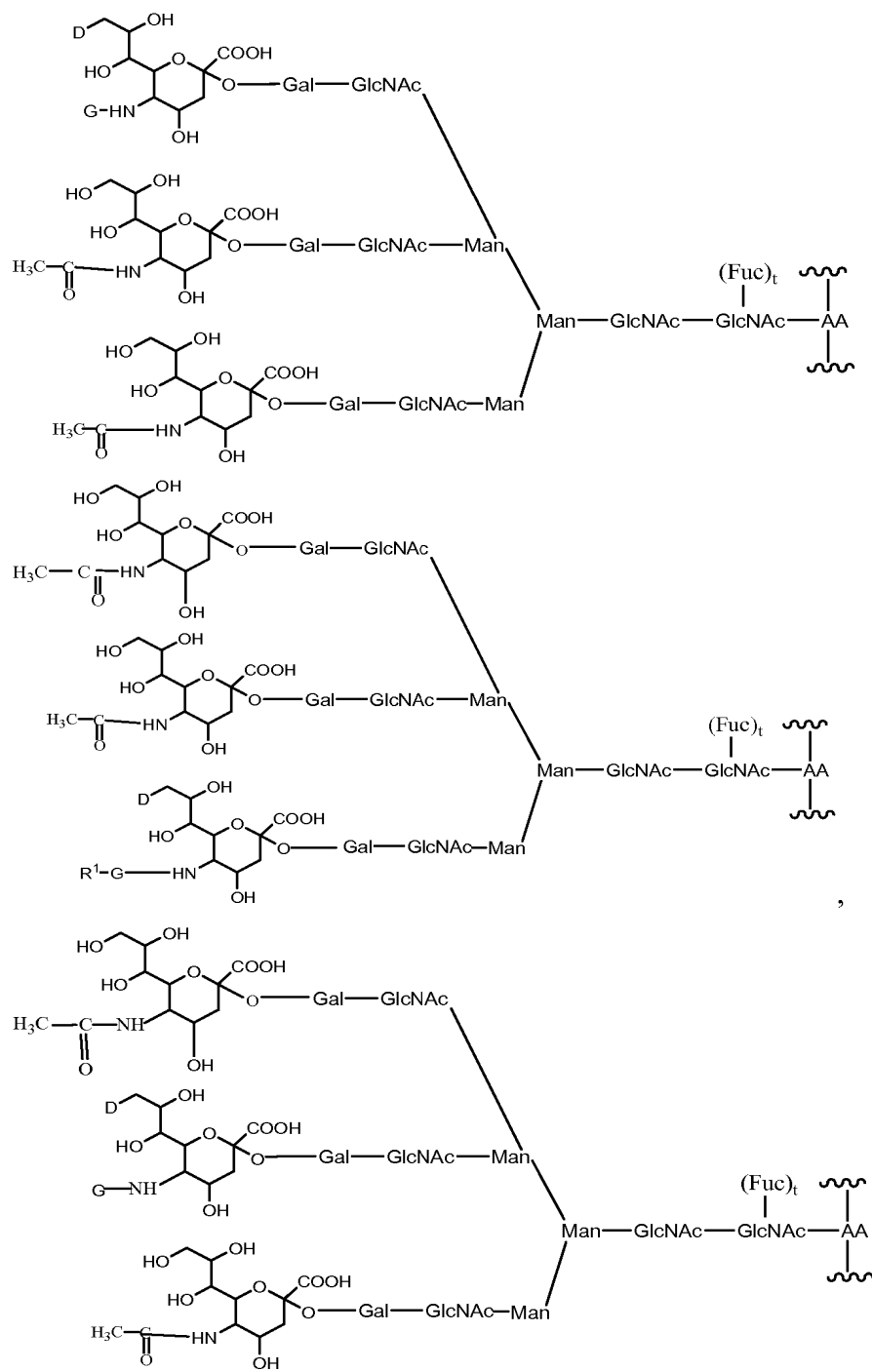
[0238] In another exemplary embodiment, the peptide conjugate comprises at least one of said glycosyl linker wherein each of said glycosyl linker has a structure which is a member independently selected from the following formulae:



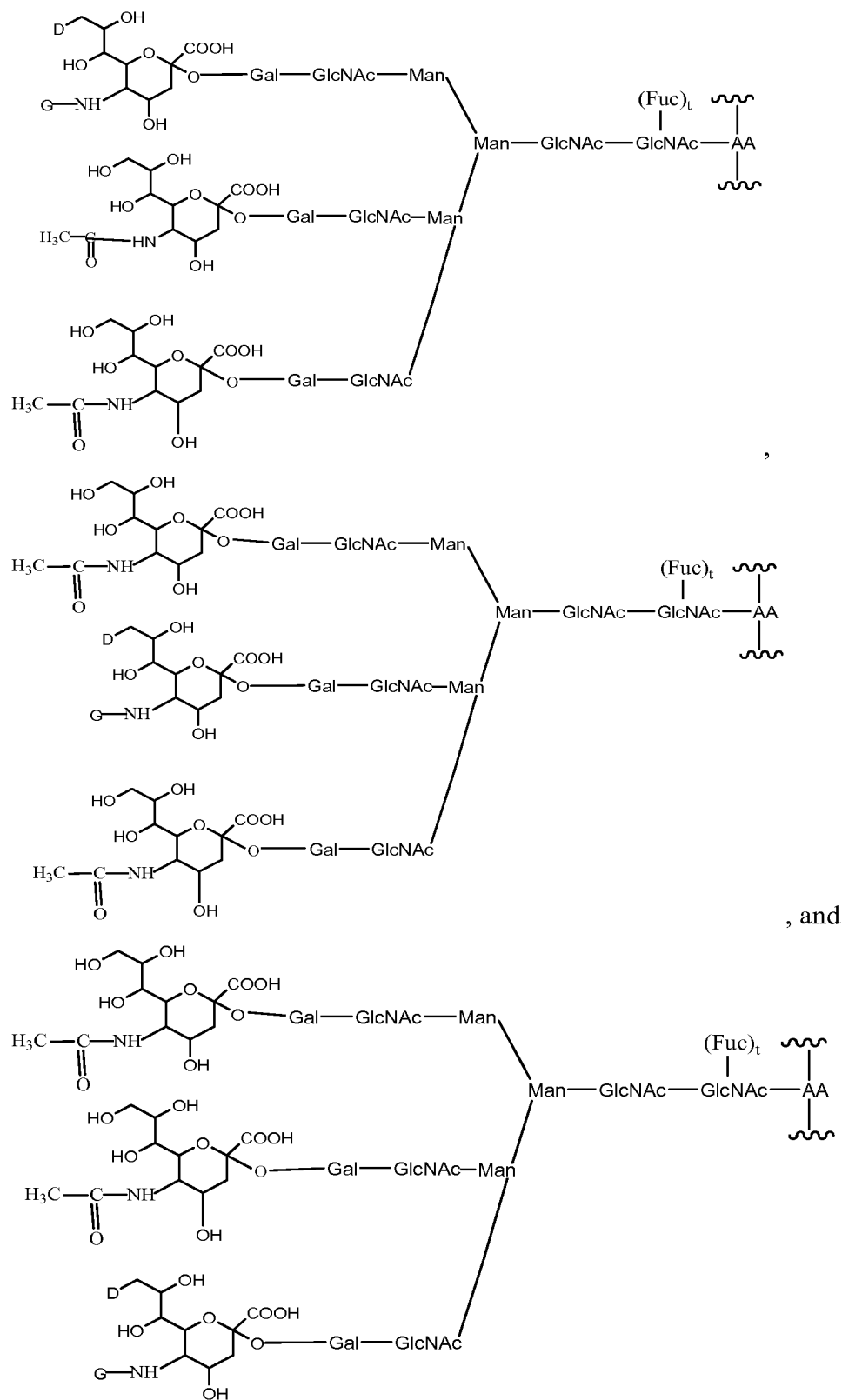


- 5 wherein D and G are as described above, AA is an amino acid residue of said peptide conjugate and t is an integer selected from 0 and 1.

[0239] In another exemplary embodiment, the peptide conjugate comprises at least one of said glycosyl linker according to a formula selected from:





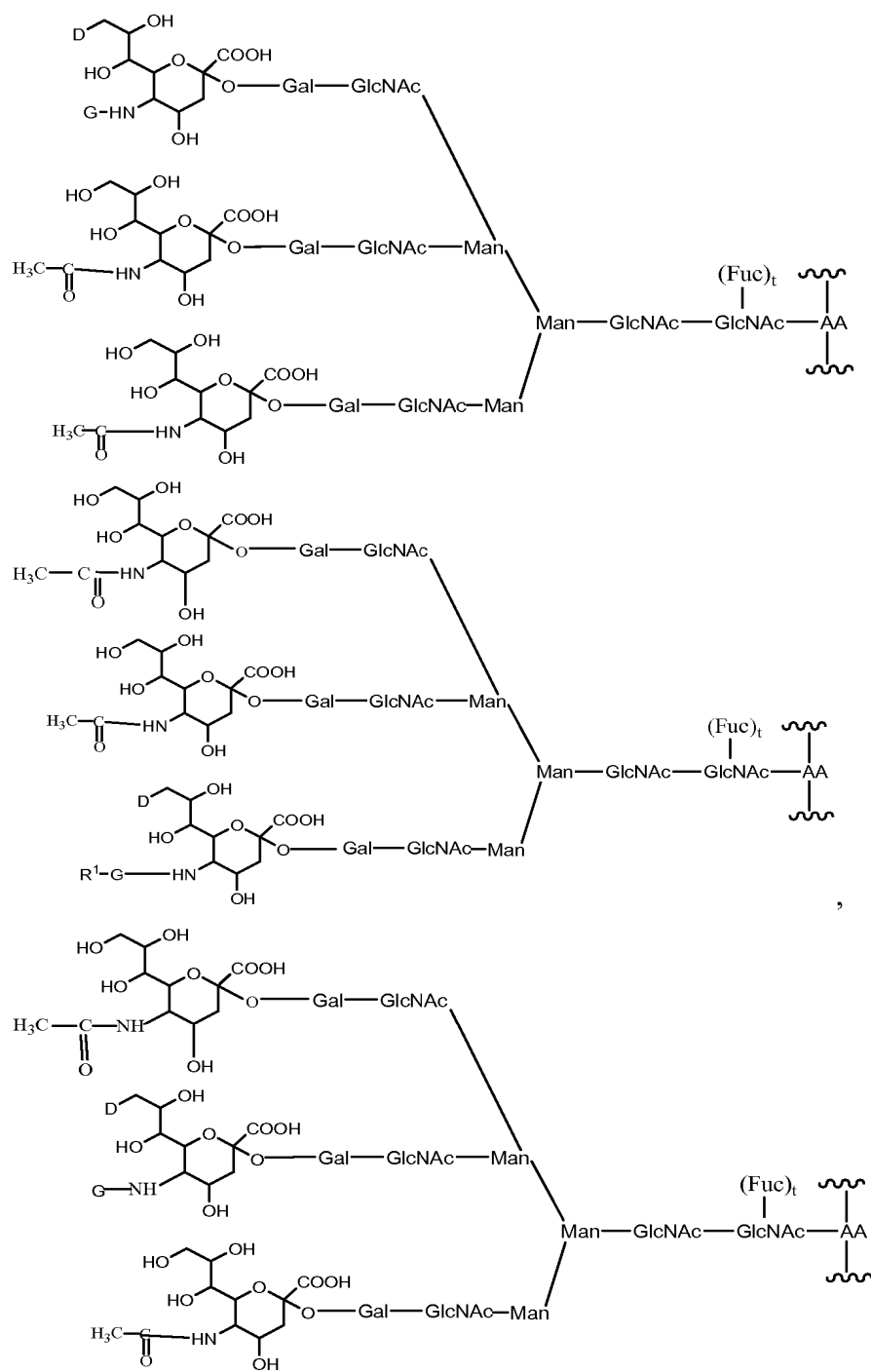


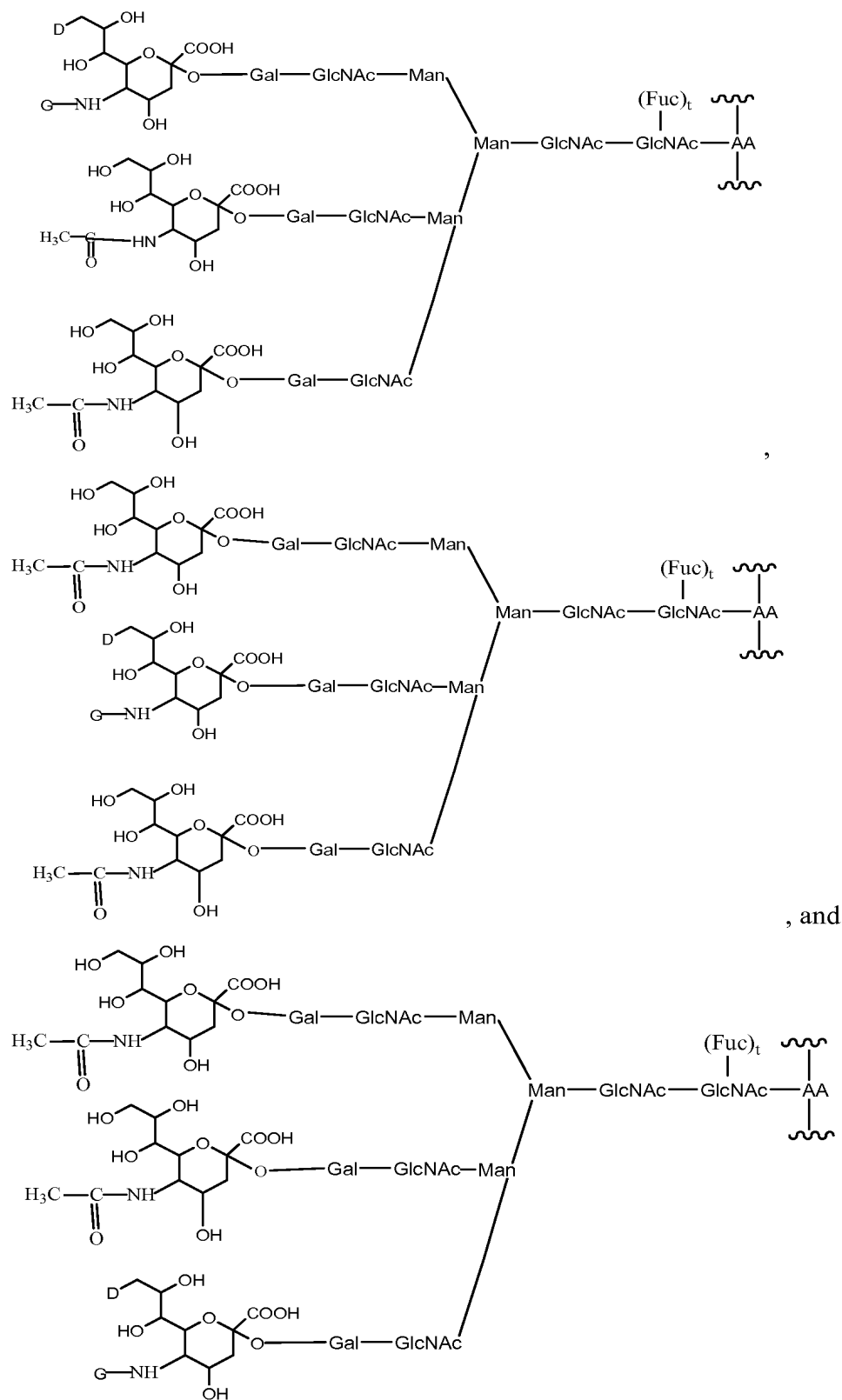
wherein D and G are as described above, AA is an amino acid residue of said peptide

5 conjugate and t is an integer selected from 0 and 1. In an exemplary embodiment, a member

selected from 0 and 2 of the sialyl moieties which do not comprise G are absent. In an exemplary embodiment, a member selected from 1 and 2 of the sialyl moieties which do not comprise G are absent.

[0240] In another exemplary embodiment, the peptide conjugate comprises at least one of said glycosyl linker according to a formula selected from:



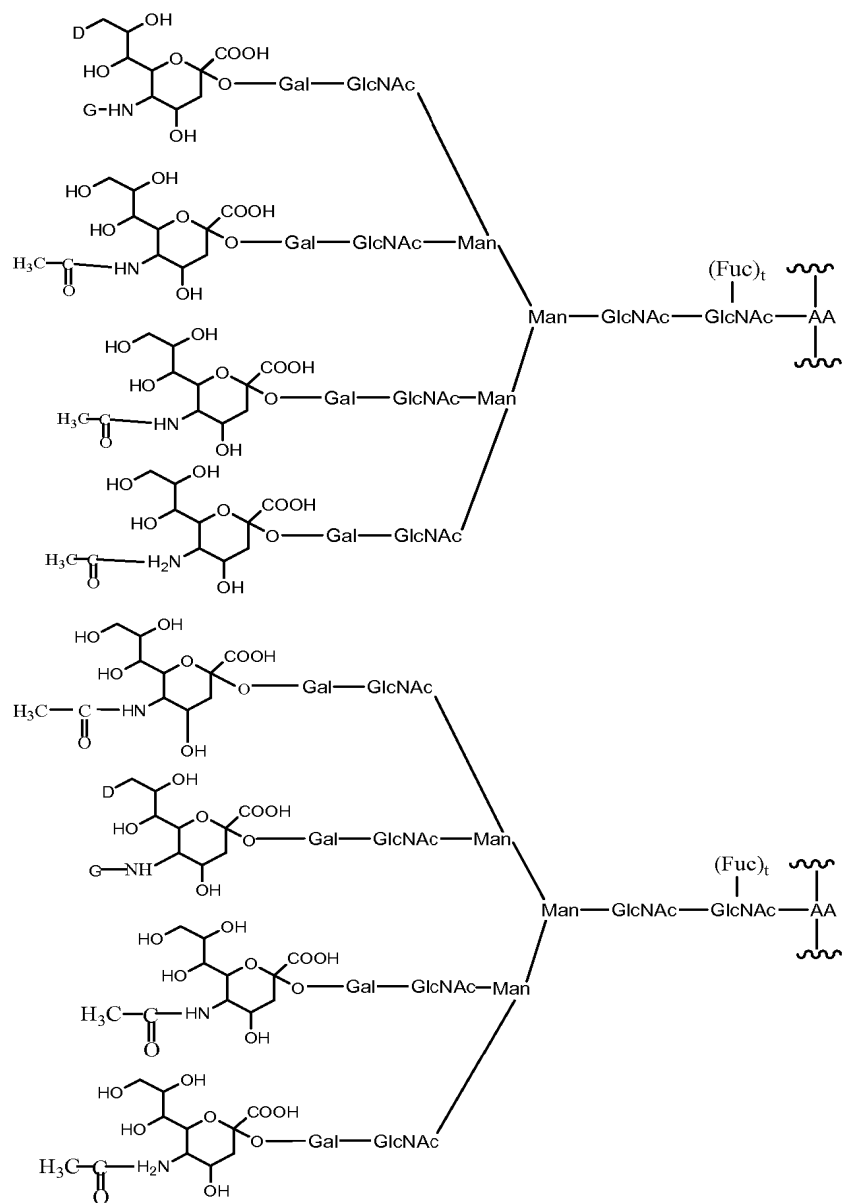


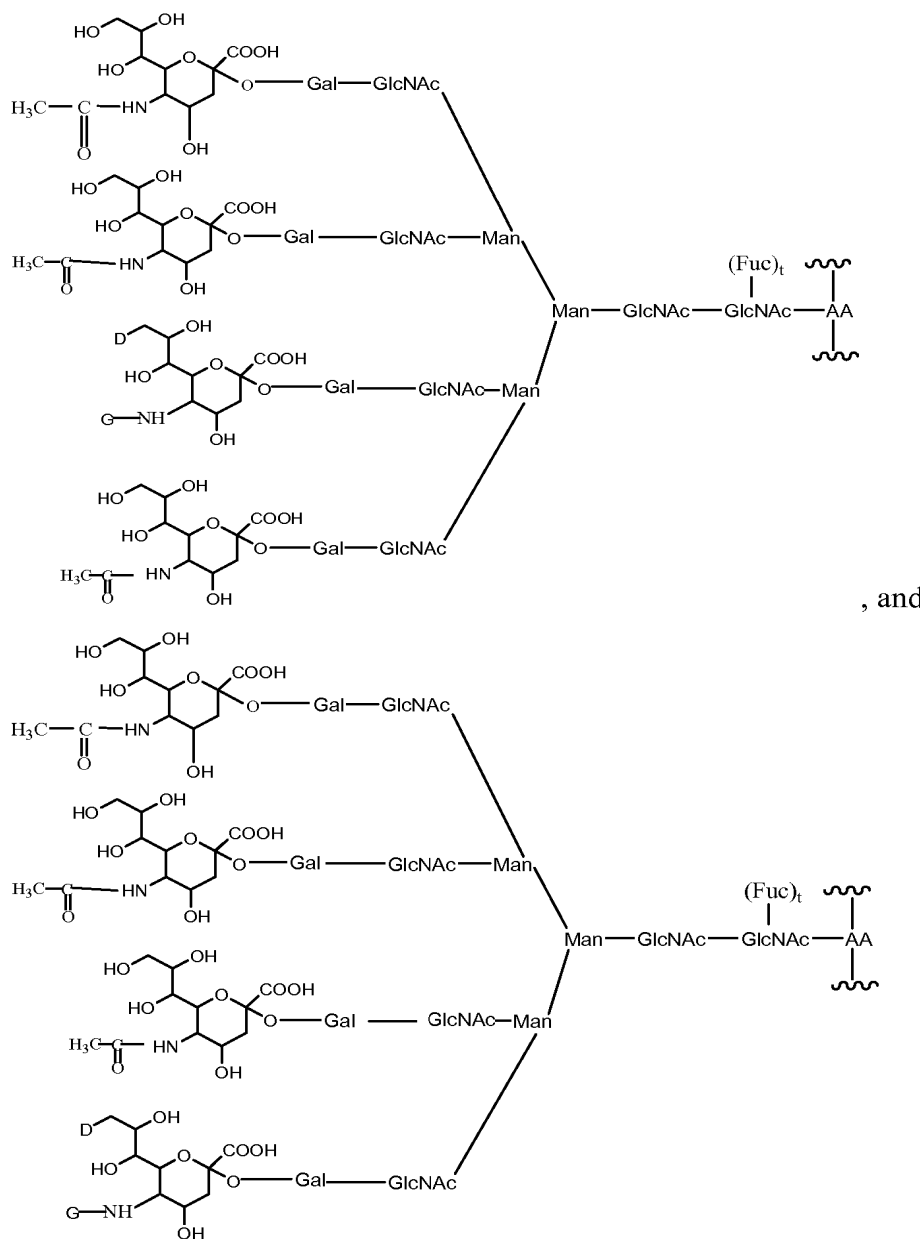
wherein D and G are as described above, AA is an amino acid residue of said peptide

5 conjugate and t is an integer selected from 0 and 1. In an exemplary embodiment, a member

selected from 0 and 2 of the sialyl moieties which do not comprise G are absent. In an exemplary embodiment, a member selected from 1 and 2 of the sialyl moieties which do not comprise G are absent.

[0241] In another exemplary embodiment, the peptide conjugate comprises at least one said glycosyl linker according to a formula selected from:





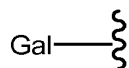
wherein D and G are as described above, AA is an amino acid residue of said peptide conjugate and t is an integer selected from 0 and 1. In an exemplary embodiment, a member  
 5 selected from 0 and 2 of the sialyl moieties which do not comprise G are absent. In an exemplary embodiment, a member selected from 1 and 2 of the sialyl moieties which do not comprise G are absent. .

**[0242]** In another exemplary embodiment, the invention provides a peptide which is produced in a suitable host. The invention also provides methods of expressing this peptide.

10 In another exemplary embodiment, the host is a mammalian expression system.

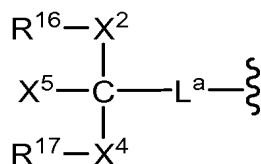
[0243] In another exemplary embodiment, the invention provides a method of treating a condition in a subject in need thereof, said condition characterized by compromised clotting potency in said subject, said method comprising the step of administering to the subject an amount of the peptide conjugate of invention, effective to ameliorate said condition in said subject. In another exemplary embodiment, the method comprises administering to said mammal an amount of the peptide conjugate produced according to the methods described herein.

[0244] In another aspect, the invention provides a method of making a peptide conjugate comprising a glycosyl linker described herein. The method comprises (a) contacting a peptide comprising the glycosyl moiety:

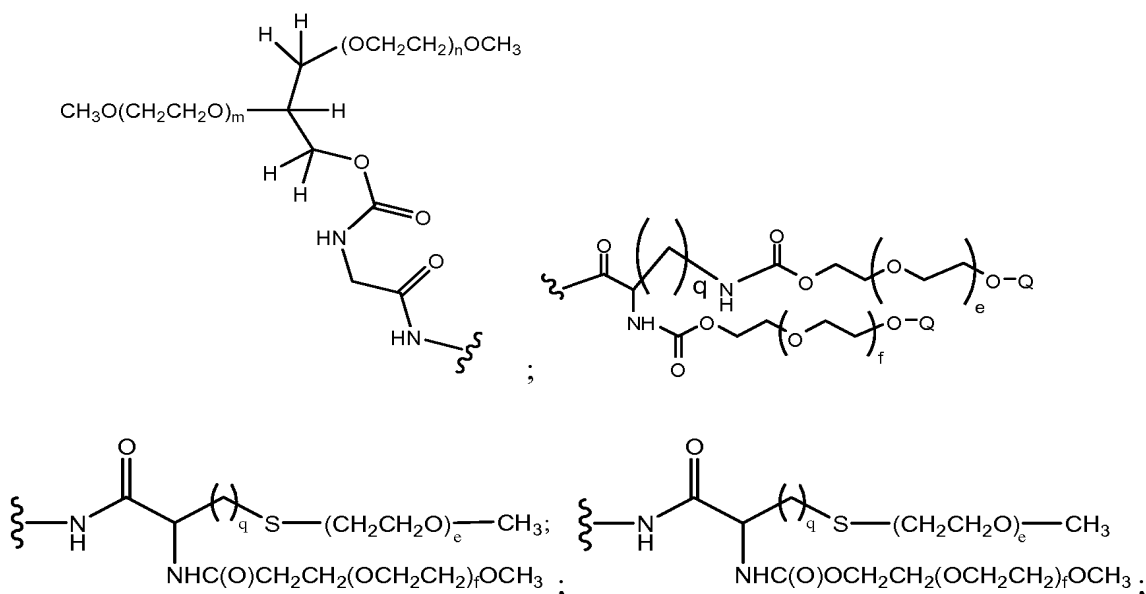


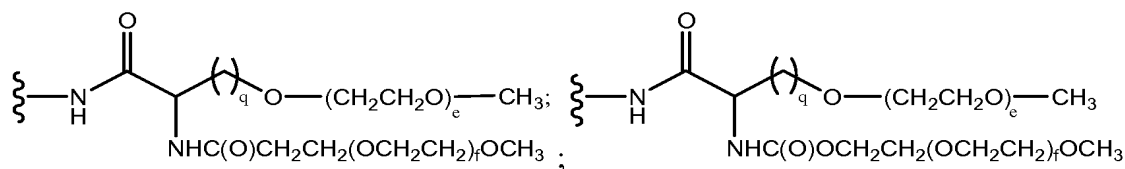
with a PEGylated nucleotide sugar described herein and an enzyme that transfers the PEGylated sugar onto the Gal of said glycosyl moiety, under conditions appropriate for said transfer.

[0245] In another exemplary embodiment, the moiety:



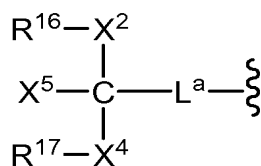
has a formula that is a member selected from:





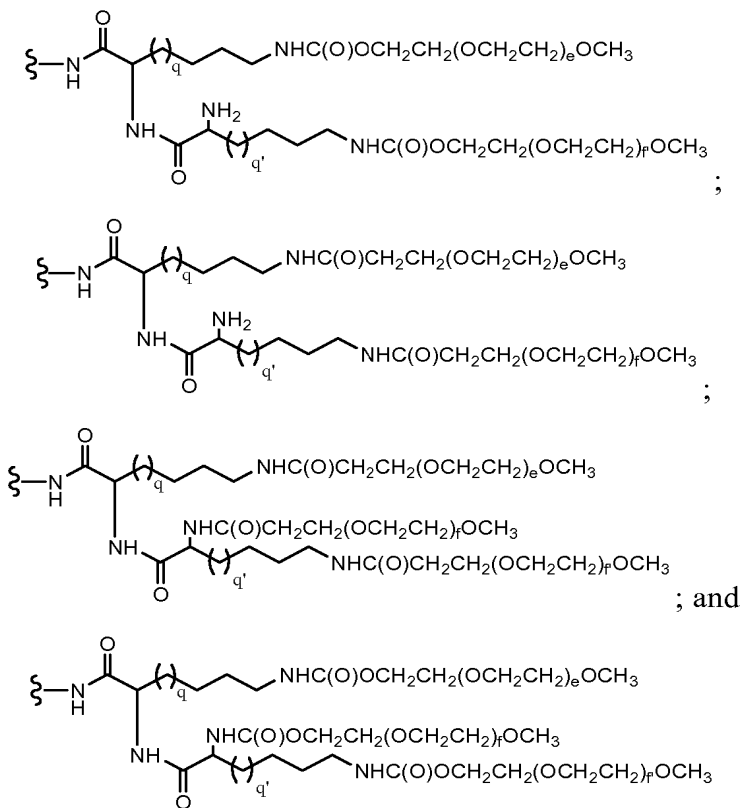
wherein e, f, m and n are integers independently selected from 1 to 2500; and q is an integer selected from 0 to 20.

[0246] In another exemplary embodiment, the moiety:



5

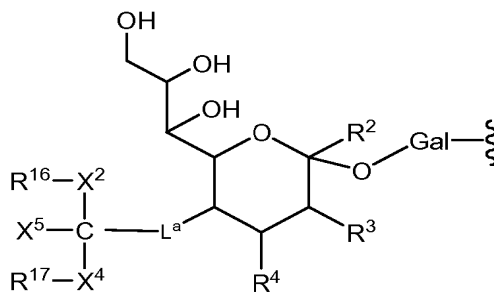
has a formula that is a member selected from:



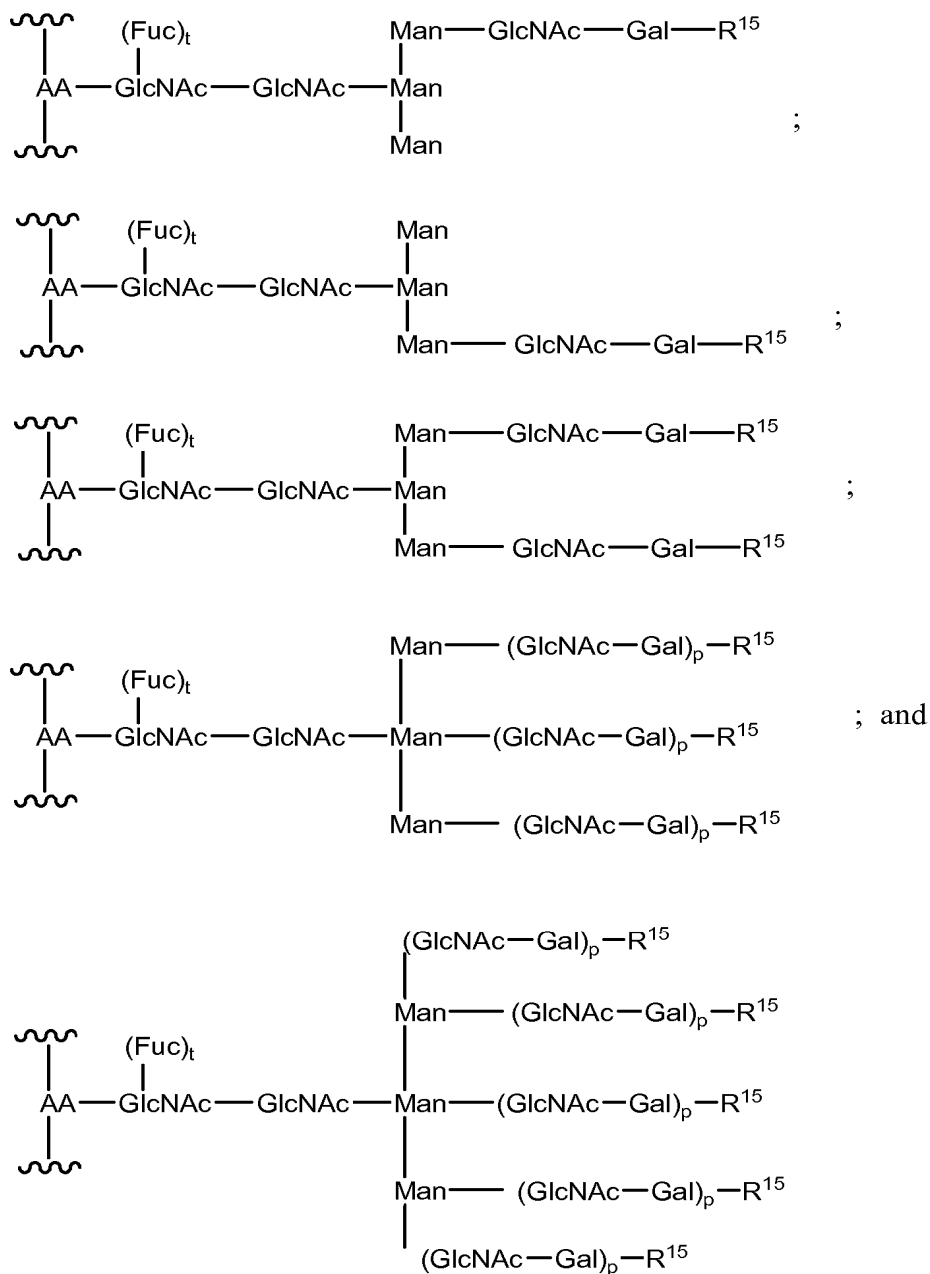
10

wherein e, f and f' are integers independently selected from 1 to 2500; and q and q' are integers independently selected from 1 to 20.

[0247] In another exemplary embodiment, the glycosyl linker comprises the formula:



[0248] In another exemplary embodiment, the peptide conjugate comprises at least one glycosyl linker having the formula:





wherein AA is an amino acid residue of said peptide; t is an integer selected from 0 and 1; and R<sup>15</sup> is the modified sialyl moiety.

[0249] In another exemplary embodiment, the method comprises, prior to step (a): (b) expressing the peptide in a suitable host.

#### 5 **II. D. iv. Water-Insoluble Polymers**

[0250] In another embodiment, analogous to those discussed above, the modified sugars include a water-insoluble polymer, rather than a water-soluble polymer. The conjugates of the invention may also include one or more water-insoluble polymers. This embodiment of the invention is illustrated by the use of the conjugate as a vehicle with which to deliver a  
10 therapeutic peptide in a controlled manner. Polymeric drug delivery systems are known in the art. See, for example, Dunn *et al.*, Eds. POLYMERIC DRUGS AND DRUG DELIVERY SYSTEMS, ACS Symposium Series Vol. 469, American Chemical Society, Washington, D.C. 1991. Those of skill in the art will appreciate that substantially any known drug delivery system is applicable to the conjugates of the present invention.

15 [0251] The motifs forth above for R<sup>1</sup>, L-R<sup>1</sup>, R<sup>15</sup>, R<sup>15'</sup> and other radicals are equally applicable to water-insoluble polymers, which may be incorporated into the linear and branched structures without limitation utilizing chemistry readily accessible to those of skill in the art.

[0252] Representative water-insoluble polymers include, but are not limited to,  
20 polyphosphazines, poly(vinyl alcohols), polyamides, polycarbonates, polyalkylenes, polyacrylamides, polyalkylene glycols, polyalkylene oxides, polyalkylene terephthalates, polyvinyl ethers, polyvinyl esters, polyvinyl halides, polyvinylpyrrolidone, polyglycolides, polysiloxanes, polyurethanes, poly(methyl methacrylate), poly(ethyl methacrylate), poly(butyl methacrylate), poly(isobutyl methacrylate), poly(hexyl methacrylate),  
25 poly(isodecyl methacrylate), poly(lauryl methacrylate), poly(phenyl methacrylate), poly(methyl acrylate), poly(isopropyl acrylate), poly(isobutyl acrylate), poly(octadecyl acrylate) polyethylene, polypropylene, poly(ethylene glycol), poly(ethylene oxide), poly(ethylene terephthalate), poly(vinyl acetate), polyvinyl chloride, polystyrene, polyvinyl pyrrolidone, pluronics and polyvinylphenol and copolymers thereof.

30 [0253] Synthetically modified natural polymers of use in conjugates of the invention include, but are not limited to, alkyl celluloses, hydroxyalkyl celluloses, cellulose ethers,

cellulose esters, and nitrocelluloses. Particularly preferred members of the broad classes of synthetically modified natural polymers include, but are not limited to, methyl cellulose, ethyl cellulose, hydroxypropyl cellulose, hydroxypropyl methyl cellulose, hydroxybutyl methyl cellulose, cellulose acetate, cellulose propionate, cellulose acetate butyrate, cellulose acetate phthalate, carboxymethyl cellulose, cellulose triacetate, cellulose sulfate sodium salt, and polymers of acrylic and methacrylic esters and alginic acid.

[0254] These and the other polymers discussed herein can be readily obtained from commercial sources such as Sigma Chemical Co. (St. Louis, MO.), Polysciences (Warrenton, PA.), Aldrich (Milwaukee, WI.), Fluka (Ronkonkoma, NY), and BioRad (Richmond, CA), or else synthesized from monomers obtained from these suppliers using standard techniques.

[0255] Representative biodegradable polymers of use in the conjugates of the invention include, but are not limited to, polylactides, polyglycolides and copolymers thereof, poly(ethylene terephthalate), poly(butyric acid), poly(valeric acid), poly(lactide-co-caprolactone), poly(lactide-co-glycolide), polyanhydrides, polyorthoesters, blends and copolymers thereof. Of particular use are compositions that form gels, such as those including collagen, pluronics and the like.

[0256] The polymers of use in the invention include "hybrid" polymers that include water-insoluble materials having within at least a portion of their structure, a bioresorbable molecule. An example of such a polymer is one that includes a water-insoluble copolymer, which has a bioresorbable region, a hydrophilic region and a plurality of crosslinkable functional groups per polymer chain.

[0257] For purposes of the present invention, "water-insoluble materials" includes materials that are substantially insoluble in water or water-containing environments. Thus, although certain regions or segments of the copolymer may be hydrophilic or even water-soluble, the polymer molecule, as a whole, does not to any substantial measure dissolve in water.

[0258] For purposes of the present invention, the term "bioresorbable molecule" includes a region that is capable of being metabolized or broken down and resorbed and/or eliminated through normal excretory routes by the body. Such metabolites or break down products are preferably substantially non-toxic to the body.

[0259] The bioresorbable region may be either hydrophobic or hydrophilic, so long as the copolymer composition as a whole is not rendered water-soluble. Thus, the bioresorbable region is selected based on the preference that the polymer, as a whole, remains water-insoluble. Accordingly, the relative properties, *i.e.*, the kinds of functional groups contained  
5 by, and the relative proportions of the bioresorbable region, and the hydrophilic region are selected to ensure that useful bioresorbable compositions remain water-insoluble.

[0260] Exemplary resorbable polymers include, for example, synthetically produced resorbable block copolymers of poly( $\alpha$ -hydroxy-carboxylic acid)/poly(oxyalkylene, (*see*, Cohn *et al.*, U.S. Patent No. 4,826,945). These copolymers are not crosslinked and are water-  
10 soluble so that the body can excrete the degraded block copolymer compositions. *See*, Younes *et al.*, *J Biomed. Mater. Res.* **21**: 1301-1316 (1987); and Cohn *et al.*, *J Biomed. Mater. Res.* **22**: 993-1009 (1988).

[0261] Presently preferred bioresorbable polymers include one or more components selected from poly(esters), poly(hydroxy acids), poly(lactones), poly(amides), poly(ester-  
15 amides), poly(amino acids), poly(anhydrides), poly(orthoesters), poly(carbonates), poly(phosphazines), poly(phosphoesters), poly(thioesters), polysaccharides and mixtures thereof. More preferably still, the bioresorbable polymer includes a poly(hydroxy) acid component. Of the poly(hydroxy) acids, polylactic acid, polyglycolic acid, polycaproic acid, polybutyric acid, polyvaleric acid and copolymers and mixtures thereof are preferred.

[0262] In addition to forming fragments that are absorbed *in vivo* ("bioresorbed"), preferred polymeric coatings for use in the methods of the invention can also form an excretable and/or metabolizable fragment.

[0263] Higher order copolymers can also be used in the present invention. For example, Casey *et al.*, U.S. Patent No. 4,438,253, which issued on March 20, 1984, discloses tri-block  
25 copolymers produced from the transesterification of poly(glycolic acid) and an hydroxyl-ended poly(alkylene glycol). Such compositions are disclosed for use as resorbable monofilament sutures. The flexibility of such compositions is controlled by the incorporation of an aromatic orthocarbonate, such as tetra-p-tolyl orthocarbonate into the copolymer structure.

[0264] Other polymers based on lactic and/or glycolic acids can also be utilized. For example, Spinu, U.S. Patent No. 5,202,413, which issued on April 13, 1993, discloses

biodegradable multi-block copolymers having sequentially ordered blocks of polylactide and/or polyglycolide produced by ring-opening polymerization of lactide and/or glycolide onto either an oligomeric diol or a diamine residue followed by chain extension with a di-functional compound, such as, a diisocyanate, diacylchloride or dichlorosilane.

5    **[0265]**    Bioresorbable regions of coatings useful in the present invention can be designed to be hydrolytically and/or enzymatically cleavable. For purposes of the present invention, "hydrolytically cleavable" refers to the susceptibility of the copolymer, especially the bioresorbable region, to hydrolysis in water or a water-containing environment. Similarly, "enzymatically cleavable" as used herein refers to the susceptibility of the copolymer,  
10    especially the bioresorbable region, to cleavage by endogenous or exogenous enzymes.

**[0266]**    When placed within the body, the hydrophilic region can be processed into excretable and/or metabolizable fragments. Thus, the hydrophilic region can include, for example, polyethers, polyalkylene oxides, polyols, poly(vinyl pyrrolidine), poly(vinyl alcohol), poly(alkyl oxazolines), polysaccharides, carbohydrates, peptides, proteins and  
15    copolymers and mixtures thereof. Furthermore, the hydrophilic region can also be, for example, a poly(alkylene) oxide. Such poly(alkylene) oxides can include, for example, poly(ethylene) oxide, poly(propylene) oxide and mixtures and copolymers thereof.

**[0267]**    Polymers that are components of hydrogels are also useful in the present invention. Hydrogels are polymeric materials that are capable of absorbing relatively large quantities of  
20    water. Examples of hydrogel forming compounds include, but are not limited to, polyacrylic acids, sodium carboxymethylcellulose, polyvinyl alcohol, polyvinyl pyrrolidine, gelatin, carrageenan and other polysaccharides, hydroxyethylenemethacrylic acid (HEMA), as well as derivatives thereof, and the like. Hydrogels can be produced that are stable, biodegradable and bioresorbable. Moreover, hydrogel compositions can include subunits that exhibit one or  
25    more of these properties.

**[0268]**    Bio-compatible hydrogel compositions whose integrity can be controlled through crosslinking are known and are presently preferred for use in the methods of the invention. For example, Hubbell *et al.*, U.S. Patent Nos. 5,410,016, which issued on April 25, 1995 and 5,529,914, which issued on June 25, 1996, disclose water-soluble systems, which are  
30    crosslinked block copolymers having a water-soluble central block segment sandwiched between two hydrolytically labile extensions. Such copolymers are further end-capped with photopolymerizable acrylate functionalities. When crosslinked, these systems become

hydrogels. The water soluble central block of such copolymers can include poly(ethylene glycol); whereas, the hydrolytically labile extensions can be a poly( $\alpha$ -hydroxy acid), such as polyglycolic acid or polylactic acid. *See, Sawhney et al., Macromolecules* **26**: 581-587 (1993).

5    **[0269]**    In another preferred embodiment, the gel is a thermoreversible gel. Thermoreversible gels including components, such as pluronics, collagen, gelatin, hyalouronic acid, polysaccharides, polyurethane hydrogel, polyurethane-urea hydrogel and combinations thereof are presently preferred.

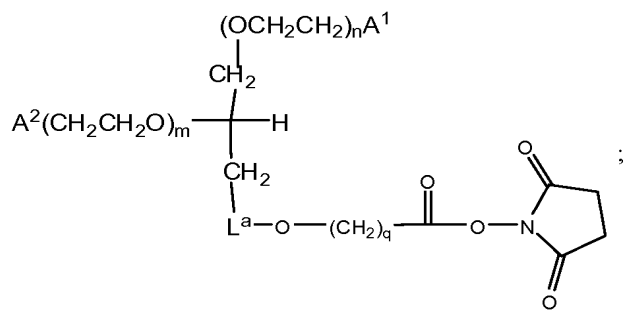
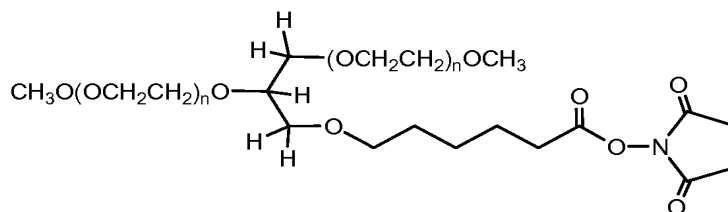
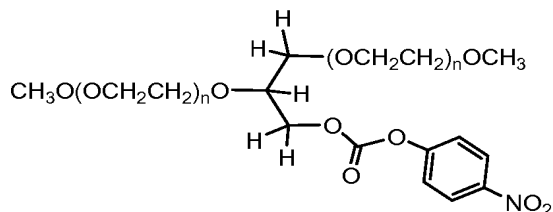
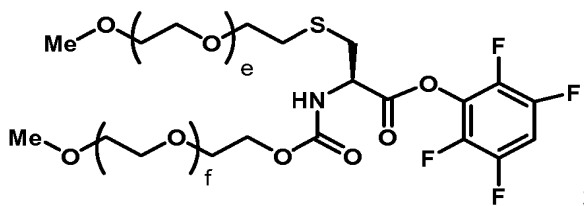
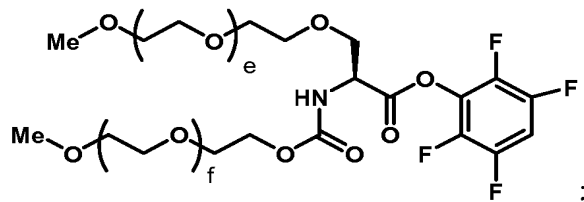
10   **[0270]**    In yet another exemplary embodiment, the conjugate of the invention includes a component of a liposome. Liposomes can be prepared according to methods known to those skilled in the art, for example, as described in Eppstein *et al.*, U.S. Patent No. 4,522,811. For example, liposome formulations may be prepared by dissolving appropriate lipid(s) (such as stearoyl phosphatidyl ethanolamine, stearoyl phosphatidyl choline, arachadoyl phosphatidyl choline, and cholesterol) in an inorganic solvent that is then evaporated, leaving behind a thin  
15   film of dried lipid on the surface of the container. An aqueous solution of the active compound or its pharmaceutically acceptable salt is then introduced into the container. The container is then swirled by hand to free lipid material from the sides of the container and to disperse lipid aggregates, thereby forming the liposomal suspension.

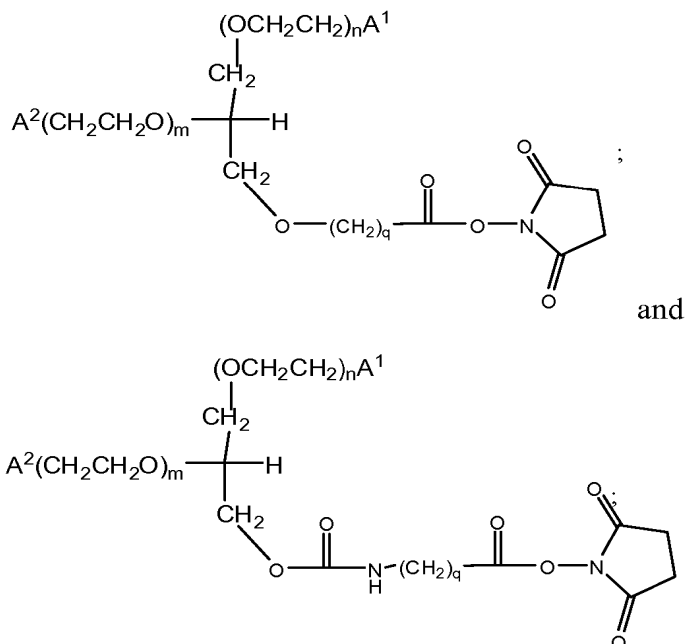
20   **[0271]**    The above-recited microparticles and methods of preparing the microparticles are offered by way of example and they are not intended to define the scope of microparticles of use in the present invention. It will be apparent to those of skill in the art that an array of microparticles, fabricated by different methods, is of use in the present invention.

25   **[0272]**    The structural formats discussed above in the context of the water-soluble polymers, both straight-chain and branched are generally applicable with respect to the water-insoluble polymers as well. Thus, for example, the cysteine, serine, dilysine, and trilysine branching cores can be functionalized with two water-insoluble polymer moieties. The methods used to produce these species are generally closely analogous to those used to produce the water-soluble polymers.

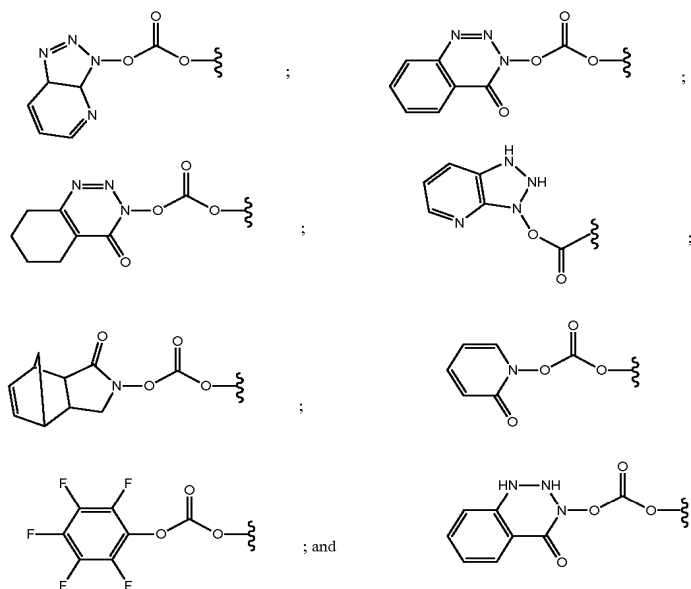
## II. D. v. *Methods of Producing the Polymeric Modifying Groups*

**[0273]** The polymeric modifying groups can be activated for reaction with a glycosyl or saccharyl moiety or an amino acid moiety. Exemplary structures of activated species (e.g., carbonates and active esters) include:





[0274] In the figure above, q is a member selected from 1-40. Other activating, or leaving groups, appropriate for activating linear and branched PEGs of use in preparing the compounds set forth herein include, but are not limited to the species:

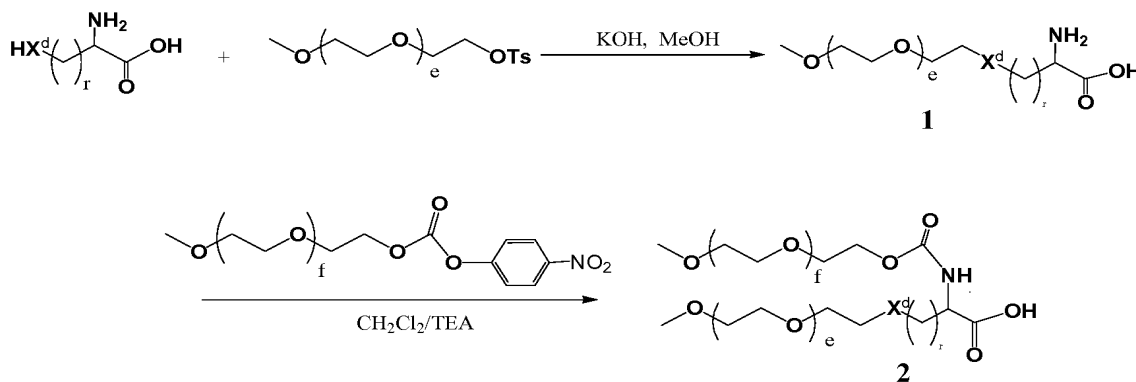


PEG molecules that are activated with these and other species and methods of making the activated PEGs are set forth in WO 04/083259.

[0275] Those of skill in the art will appreciate that one or more of the m-PEG arms of the branched polymers shown above can be replaced by a PEG moiety with a different terminus, e.g., OH, COOH, NH<sub>2</sub>, C<sub>2</sub>-C<sub>10</sub>-alkyl, etc. Moreover, the structures above are readily

modified by inserting alkyl linkers (or removing carbon atoms) between the  $\alpha$ -carbon atom and the functional group of the amino acid side chain. Thus, "homo" derivatives and higher homologues, as well as lower homologues are within the scope of cores for branched PEGs of use in the present invention.

- 5 [0276] The branched PEG species set forth herein are readily prepared by methods such as that set forth in the scheme below:



- in which  $X^d$  is O or S and  $r$  is an integer from 1 to 5. The indices  $e$  and  $f$  are independently selected integers from 1 to 2500. In an exemplary embodiment, one or both of these indices are selected such that the polymer is about 5 kD, 10 kD, 15 kD, 20 kD, 25 kD, 30 kD, 35 kD, or 40 kD in molecular weight. PEG of a larger molecular weight can also be used in the present invention, including up to about 200 kD, such as at least about 180 kD, about 160 kD, about 140 kD, about 120 kD, about 100 kD, about 90 kD, about 80 kD, and about 70 kD. In certain embodiments the molecular weight of PEG is about 80 kD. In other embodiments, the molecular weight of PEG is at least about 200 kD, at least about 180 kD, at least about 160 kD, or at least about 140 kD.

- [0277] Thus, according to this scheme, a natural or unnatural amino acid is contacted with an activated m-PEG derivative, in this case the tosylate, forming **1** by alkylating the side-chain heteroatom  $X^d$ . The mono-functionalized m-PEG amino acid is submitted to N-acylation conditions with a reactive m-PEG derivative, thereby assembling branched m-PEG **2**. As one of skill will appreciate, the tosylate leaving group can be replaced with any suitable leaving group, e.g., halogen, mesylate, triflate, etc. Similarly, the reactive carbonate utilized to acylate the amine can be replaced with an active ester, e.g., N-hydroxysuccinimide, etc., or the acid can be activated *in situ* using a dehydrating agent such as dicyclohexylcarbodiimide, carbonyldiimidazole, etc.



[0278] In other exemplary embodiments, the urea moiety is replaced by a group such as an amide.

***II. E. Homodisperse Peptide Conjugate Compositions of Matter***

[0279] In addition to providing peptide conjugates that are formed through a chemically

5 or enzymatically added glycosyl linking group, the present invention provides compositions of matter comprising peptide conjugates that are highly homogenous in their substitution patterns. Using the methods of the invention, it is possible to form peptide conjugates in which substantial proportion of the glycosyl linking groups and glycosyl moieties across a population of peptide conjugates are attached to a structurally identical amino acid or

10 glycosyl residue. Thus, in a second aspect, the invention provides a peptide conjugate having a population of water-soluble polymer moieties, which are covalently bound to the peptide through a glycosyl linking group, e.g., an intact glycosyl linking group. In an exemplary peptide conjugate of the invention, essentially each member of the water soluble polymer population is bound via the glycosyl linking group to a glycosyl residue of the peptide, and  
15 each glycosyl residue of the peptide to which the glycosyl linking group is attached has the same structure.

[0280] The present invention also provides conjugates analogous to those described above in which the peptide is conjugated to a modifying group, e.g. therapeutic moiety, diagnostic moiety, targeting moiety, toxin moiety or the like via a glycosyl linking group.

20 Each of the above-recited modifying groups can be a small molecule, natural polymer (e.g., polypeptide) or synthetic polymer. When the modifying group is attached to a sialic acid, it is generally preferred that the modifying group is substantially non-fluorescent.

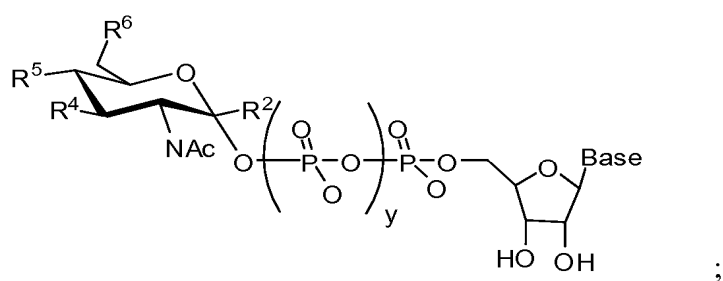
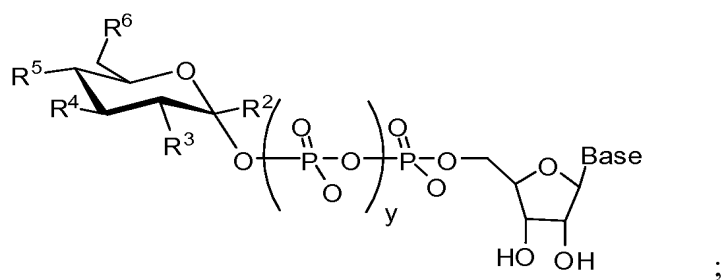
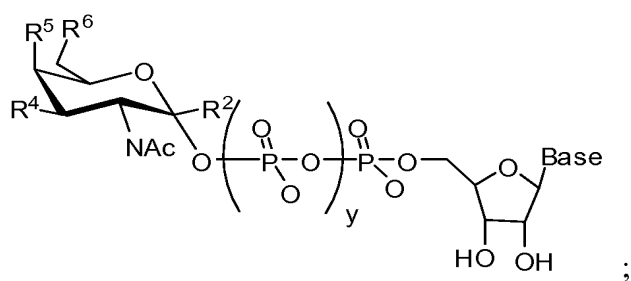
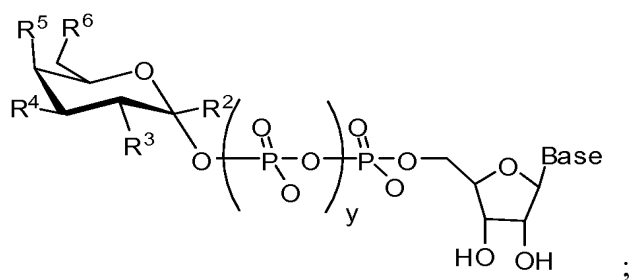
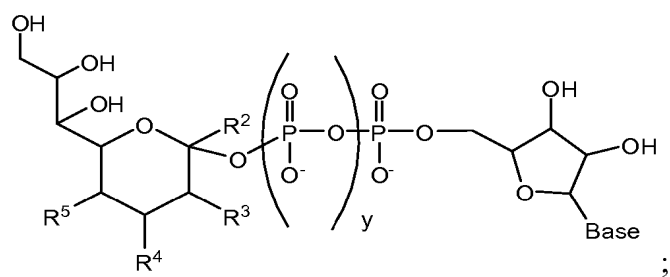
[0281] In an exemplary embodiment, the peptides of the invention include at least one O-linked or N-linked glycosylation site, which is glycosylated with a modified sugar that  
25 includes a polymeric modifying group, e.g., a PEG moiety. In an exemplary embodiment, the PEG is covalently attached to the peptide via an intact glycosyl linking group, or via a non-glycosyl linker, e.g., substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl. The glycosyl linking group is covalently attached to either an amino acid residue or a glycosyl residue of the peptide. Alternatively, the glycosyl linking group is attached to  
30 one or more glycosyl units of a glycopeptide. The invention also provides conjugates in which a glycosyl linking group is attached to both an amino acid residue and a glycosyl residue.

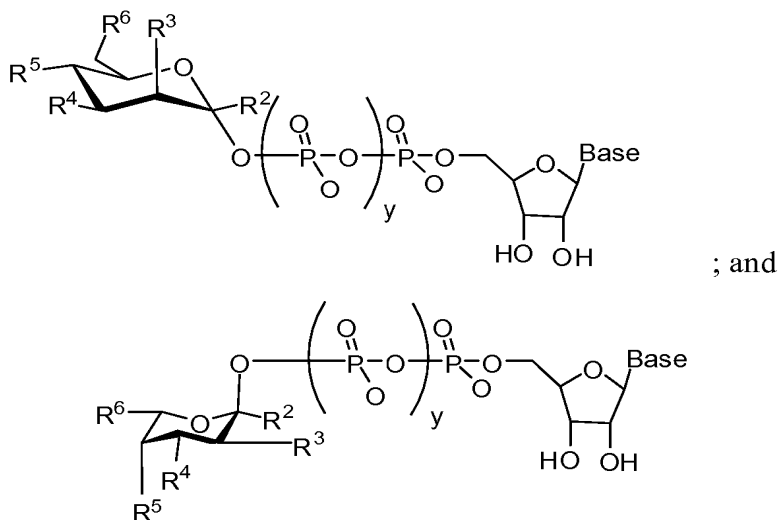
[0282] The glycans on the peptides of the invention generally correspond to those found on a peptide that is produced by mammalian (BHK, CHO) cells or insect (e.g., Sf-9) cells, following remodeling according to the methods set forth herein. For example insect-derived peptide that is expressed with a tri-mannosyl core is subsequently contacted with a GlcNAc donor and a GlcNAc transferase and a Gal donor and a Gal transferase. Appending GlcNAc and Gal to the tri-mannosyl core is accomplished in either two steps or a single step. A modified sialic acid is added to at least one branch of the glycosyl moiety as discussed herein. Those Gal moieties that are not functionalized with the modified sialic acid are optionally “capped” by reaction with a sialic acid donor in the presence of a sialyl transferase.

[0283] In an exemplary embodiment, at least 60% of terminal Gal moieties in a population of peptides is capped with sialic acid, preferably at least 70%, more preferably, at least 80%, still more preferably at least 90% and even more preferably at least 95%, 96%, 97%, 98% or 99% are capped with sialic acid.

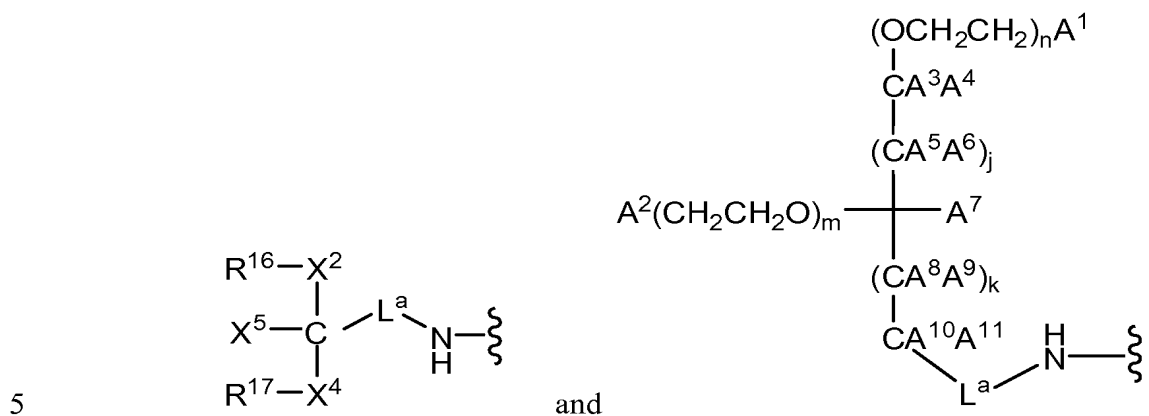
## ***II. F.      Nucleotide Sugars***

[0284] In another aspect of the invention, the invention also provides sugar nucleotides. Exemplary species according to this embodiment include:



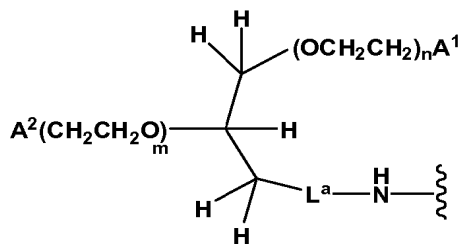


wherein  $y$  is an integer selected from 0 to 2 and at least one of  $R^2$ ,  $R^3$ ,  $R^4$ ,  $R^5$  or  $R^6$  has a structure which is a member selected from



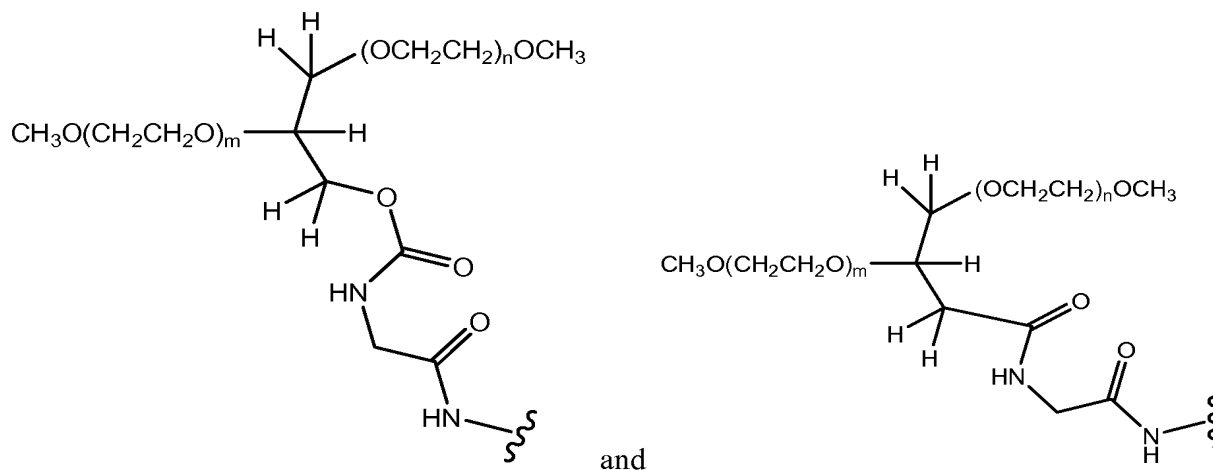
in which the variables are as described above.

**[0285]** In an exemplary embodiment, at least one of  $R^2$ ,  $R^3$ ,  $R^4$ ,  $R^5$  or  $R^6$  has a structure according to the following formula:



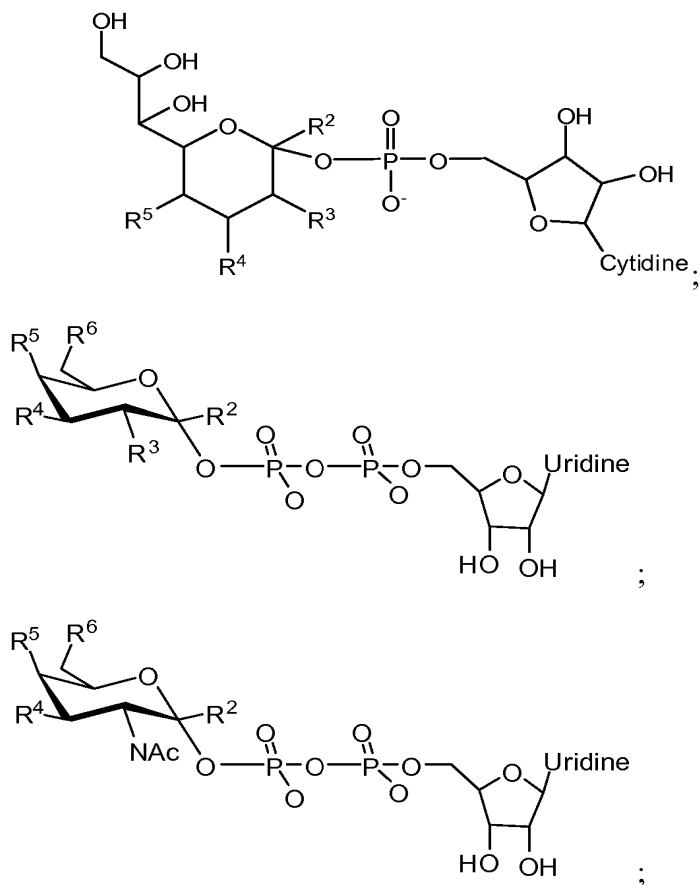
10 In an exemplary embodiment,  $A^1$  and  $A^2$  are each selected from  $-OH$  and  $-OCH_3$ .

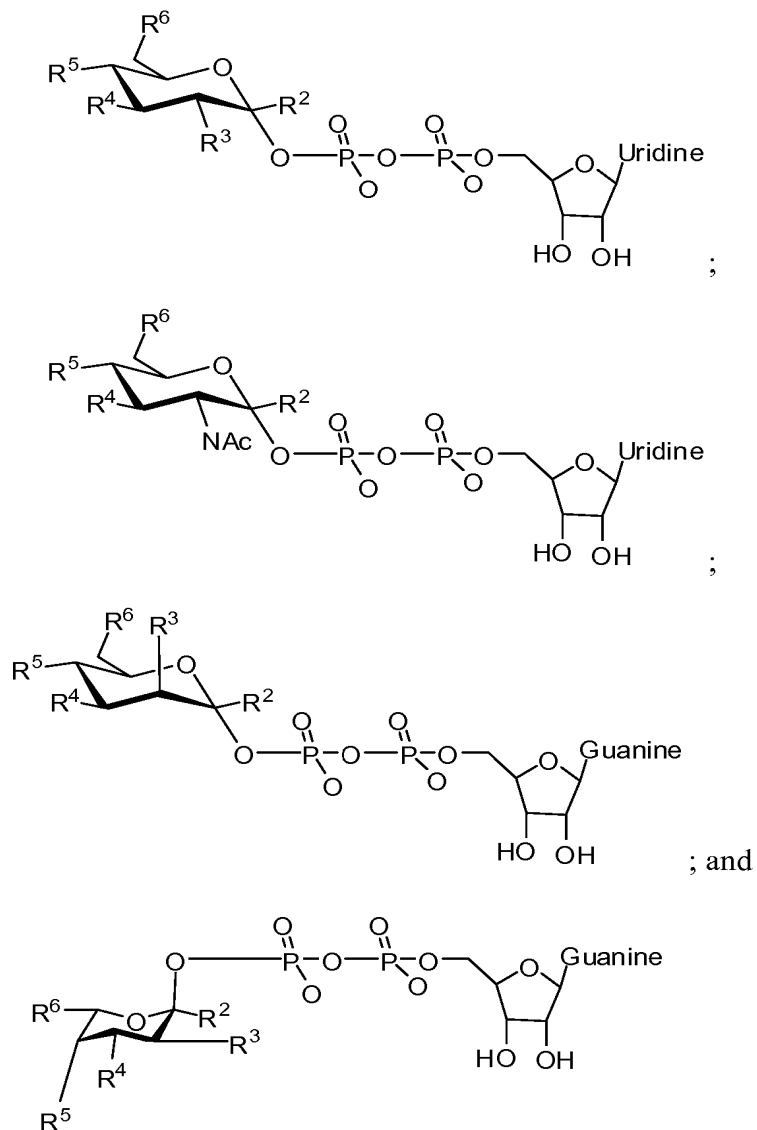
[0286] Exemplary polymeric modifying groups according to this embodiment include the moiety:



[0287] In an exemplary embodiment, only one of  $R^2$ ,  $R^3$ ,  $R^4$ ,  $R^5$  or  $R^6$  has a structure which includes the modifying groups described above.

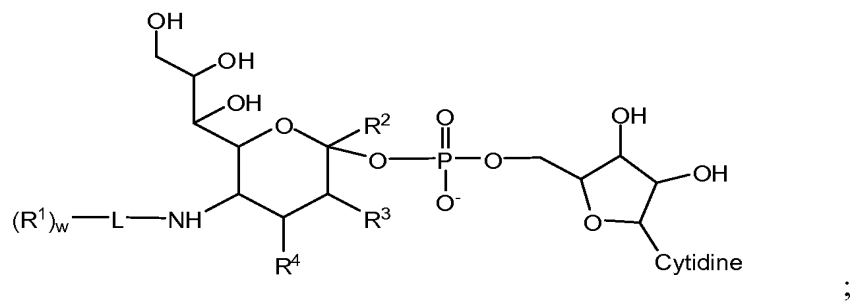
[0288] In another exemplary embodiment, species according to this embodiment include:

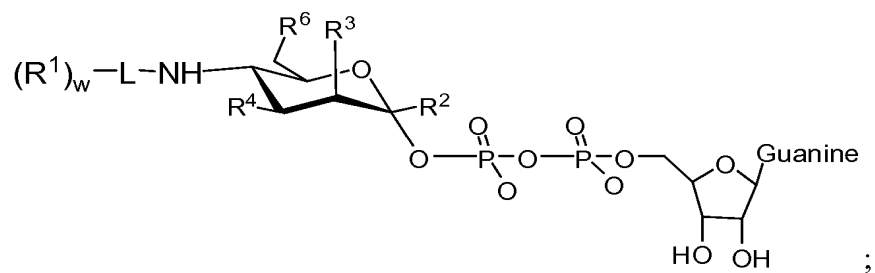
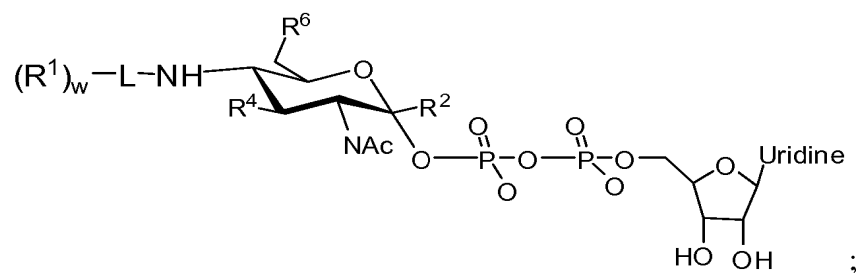
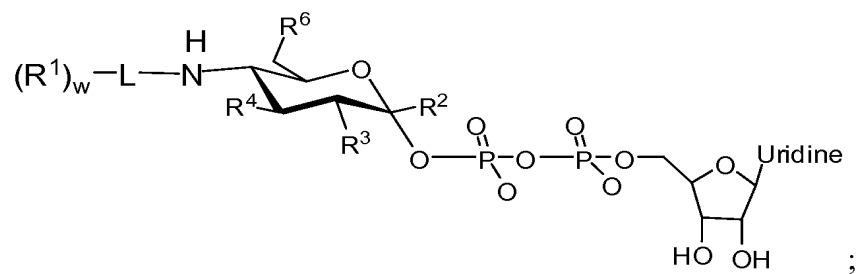
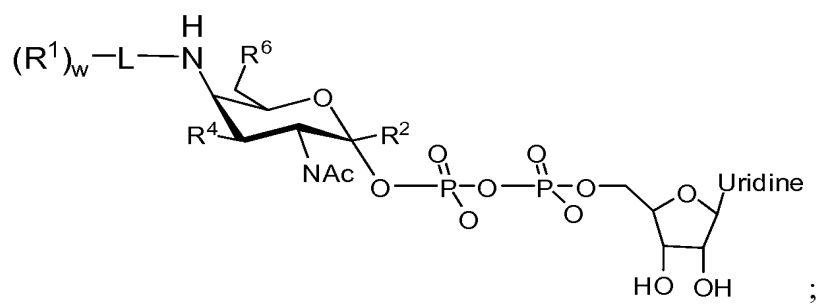
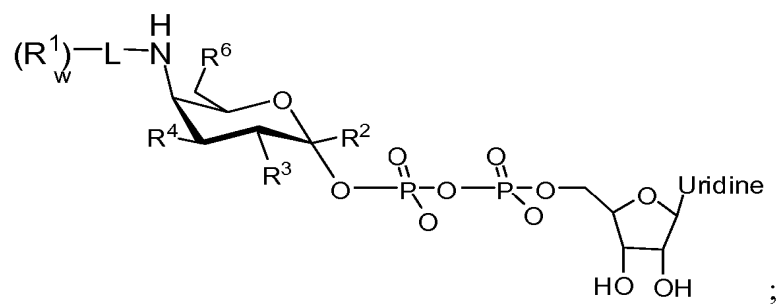


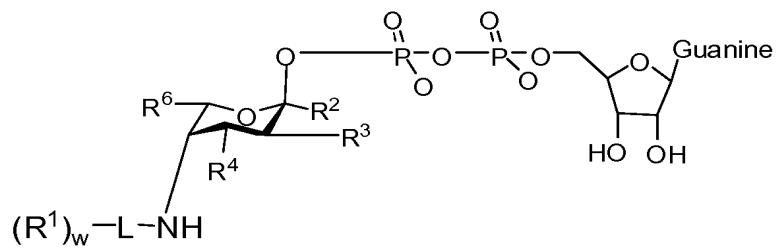


5 wherein the variables are as described above.

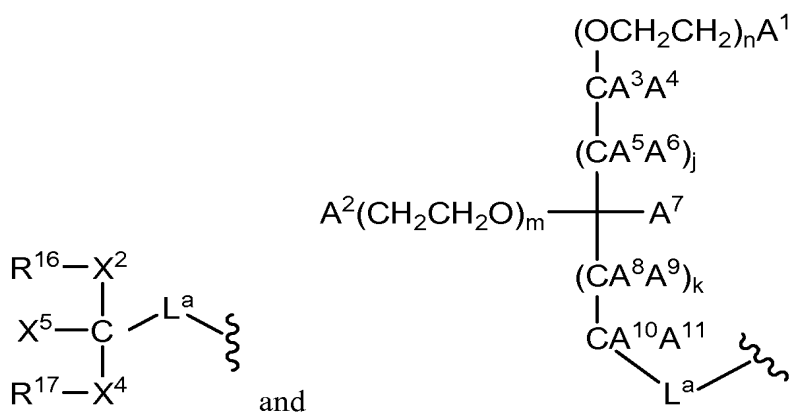
[0289] In another exemplary embodiment, species according to this embodiment include:





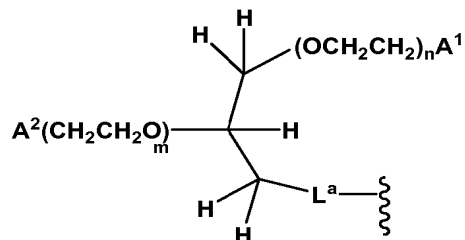


in which  $L-(R^1)_w$  is a member selected from



in which the variables are as described above.

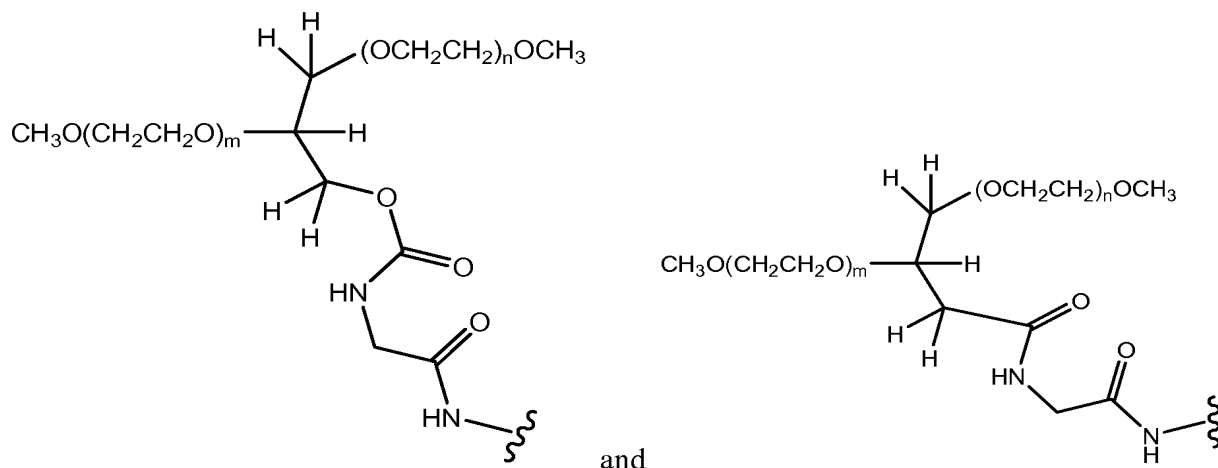
- 5 [0290] In an exemplary embodiment,  $L-(R^1)_w$  has a structure according to the following formula:



In an exemplary embodiment,  $A^1$  and  $A^2$  are each selected from  $-OH$  and  $-OCH_3$ .

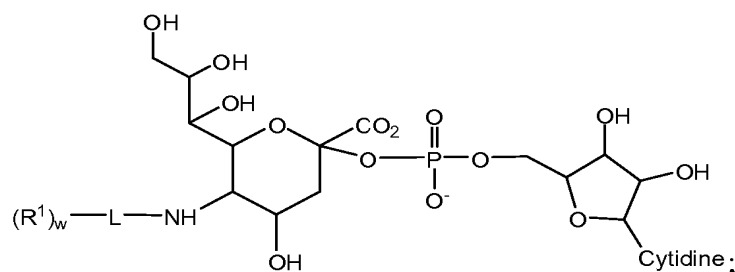


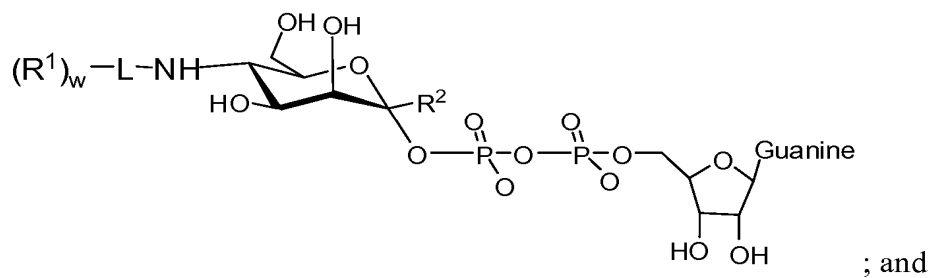
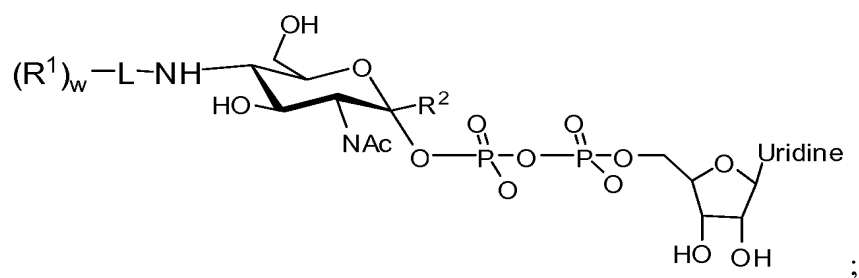
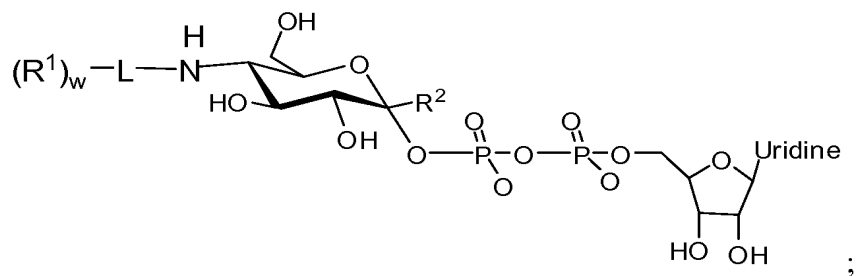
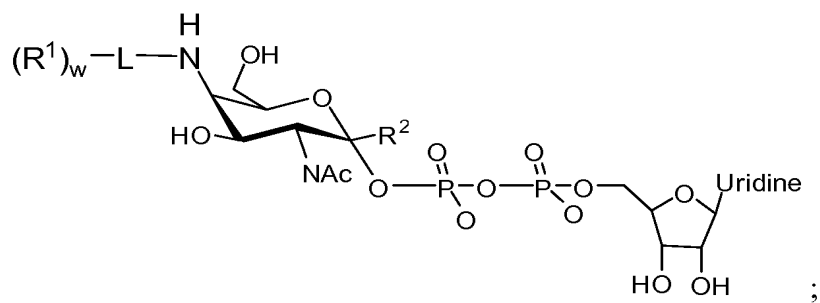
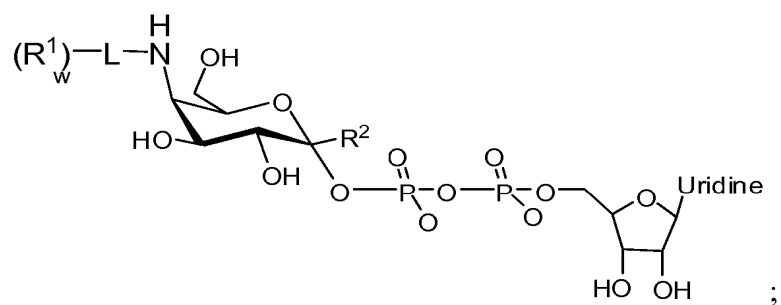
[0291] Exemplary polymeric modifying groups according to this embodiment include the moiety:

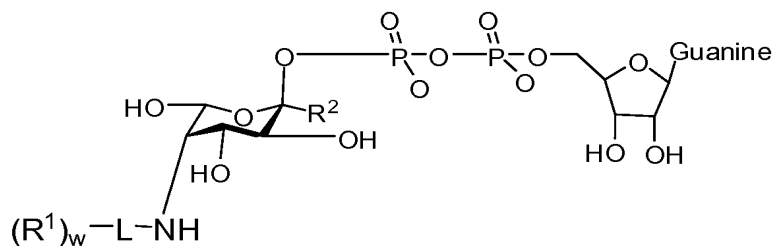


In an exemplary embodiment, m and n are integers independently selected from about 1 to about 1000. In an exemplary embodiment, m and n are integers independently selected from about 1 to about 500. In an exemplary embodiment, m and n are integers independently selected from about 1 to about 70, about 70 to about 150, about 150 to about 250, about 250 to about 375 and about 375 to about 500. In an exemplary embodiment, m and n are integers independently selected from about 10 to about 35, about 45 to about 65, about 95 to about 130, about 210 to about 240, about 310 to about 370 and about 420 to about 480. In an exemplary embodiment, m and n are integers selected from about 15 to about 30. In an exemplary embodiment, m and n are integers selected from about 50 to about 65. In an exemplary embodiment, m and n are integers selected from about 100 to about 130. In an exemplary embodiment, m and n are integers selected from about 210 to about 240. In an exemplary embodiment, m and n are integers selected from about 310 to about 370. In an exemplary embodiment, m and n are integers selected from about 430 to about 470.

[0292] In another exemplary embodiment, species according to this embodiment include:

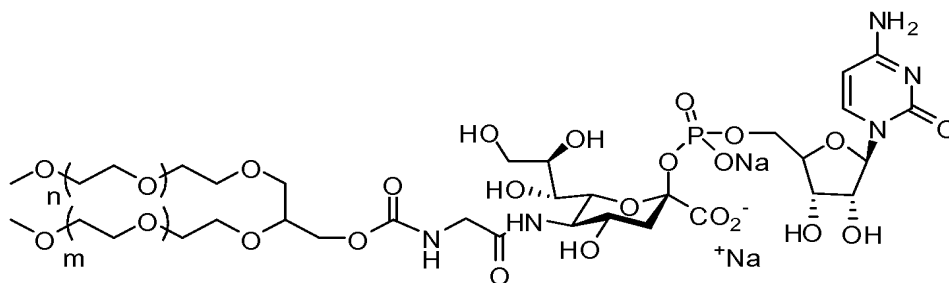




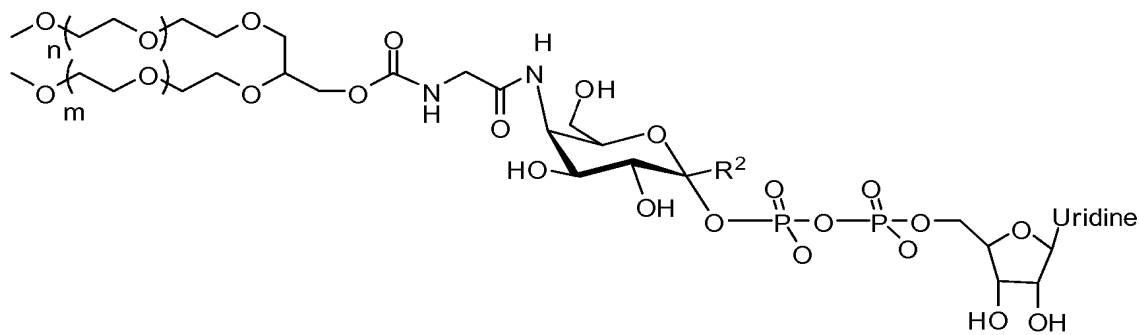


wherein the variables are as described above.

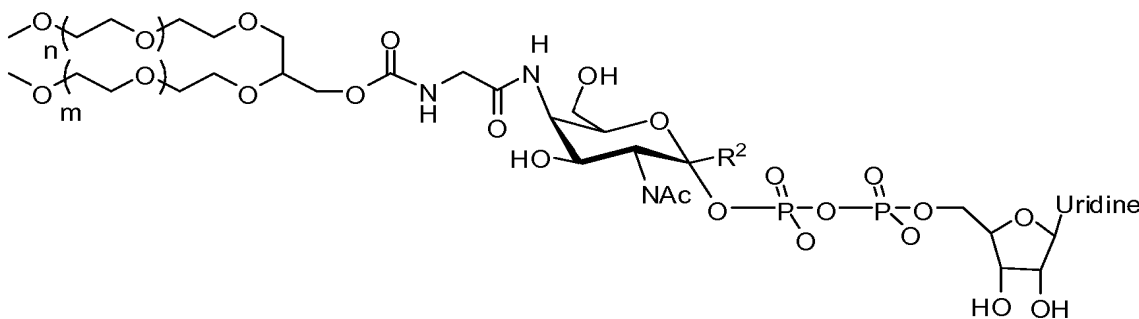
[0293] In another exemplary embodiment, species according to this embodiment include:



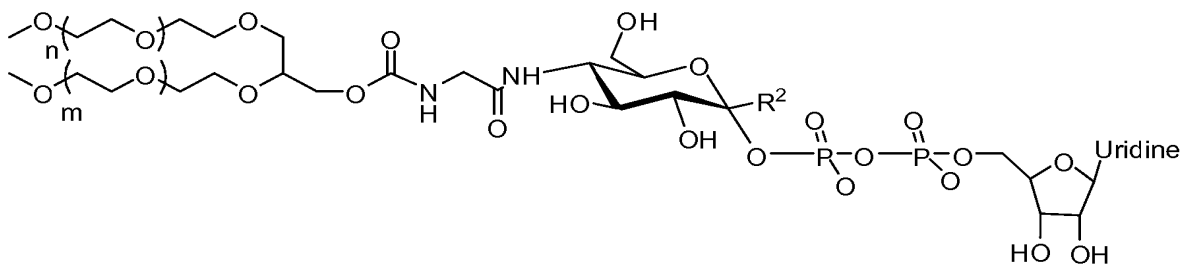
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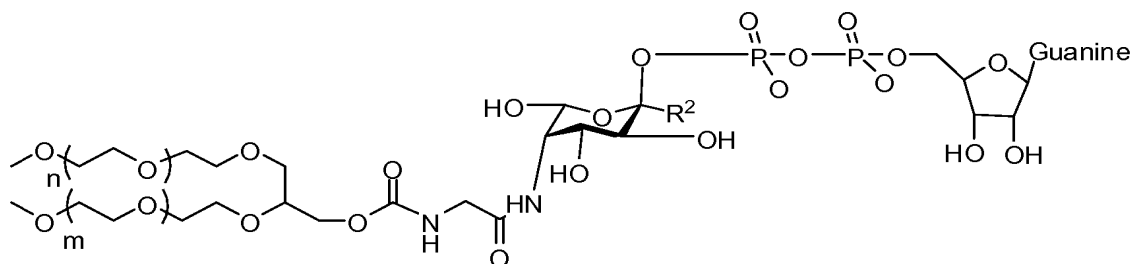
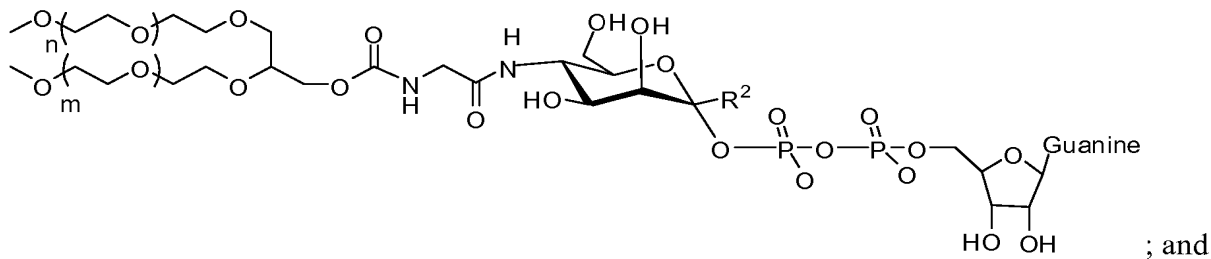
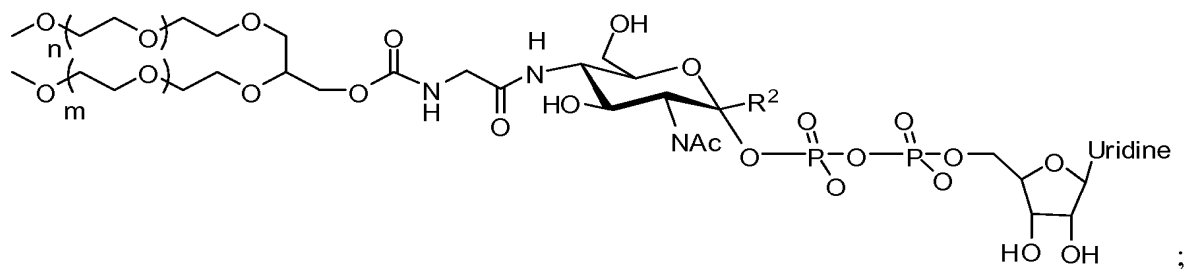
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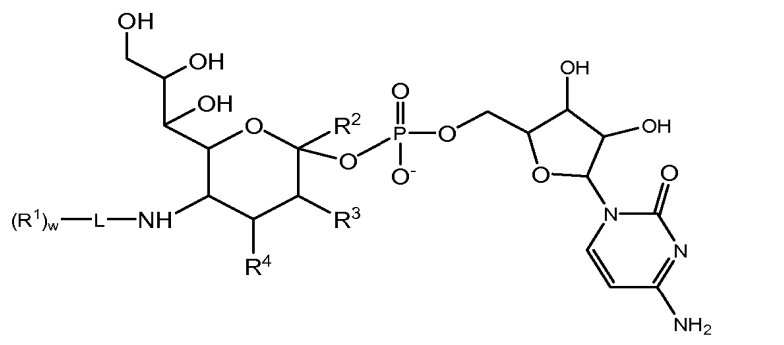


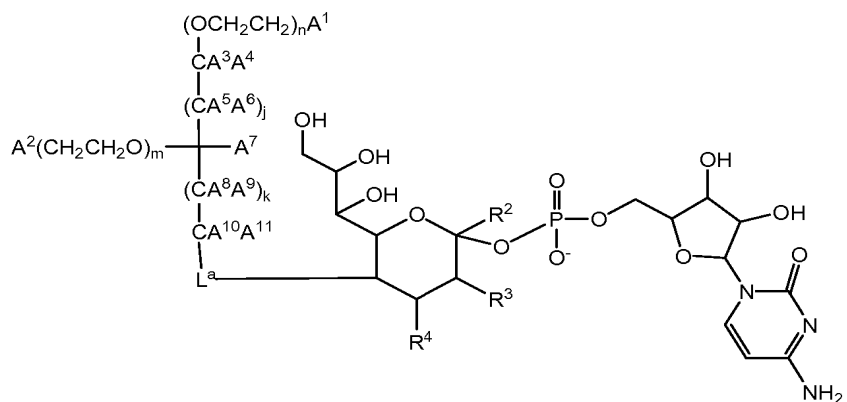
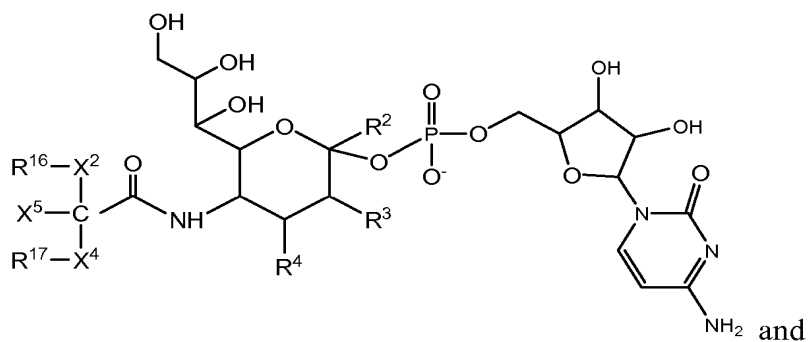
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wherein the variables are as described above.

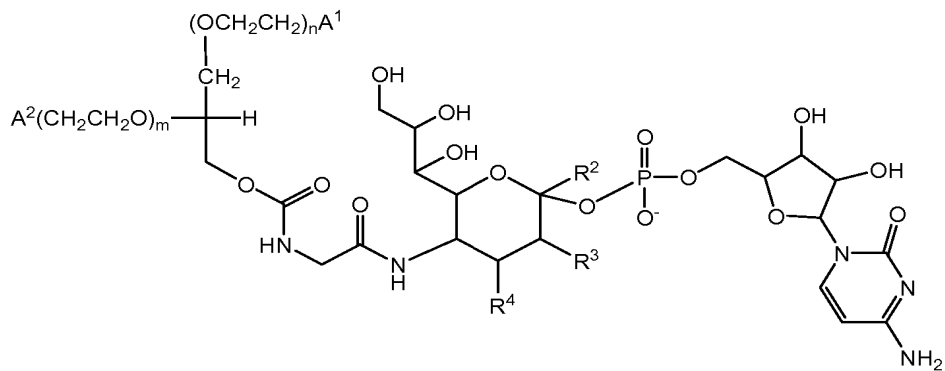
- 5 [0294] In another exemplary embodiment, the nucleotide sugars have a formula which is a member selected from:





wherein the variables are as described above.

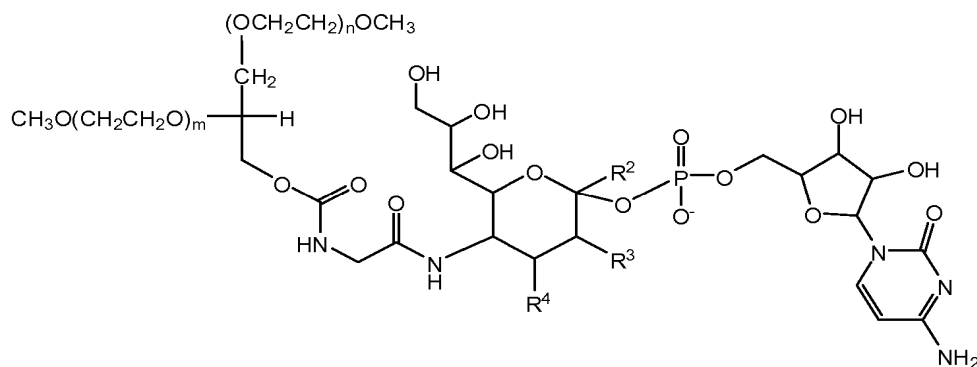
[0295] An exemplary nucleotide sugar according to this embodiment has the structure:



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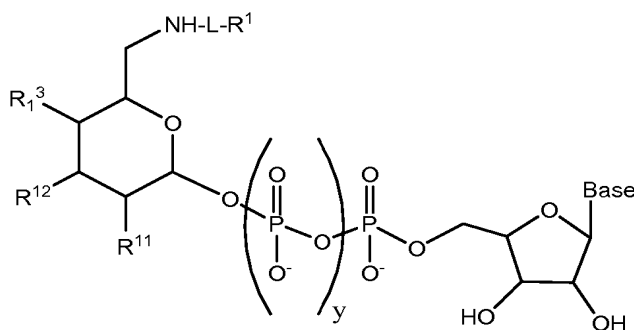
wherein the variables are as described above.

[0296] An exemplary nucleotide sugar according to this embodiment has the structure:



wherein the variables are as described above.

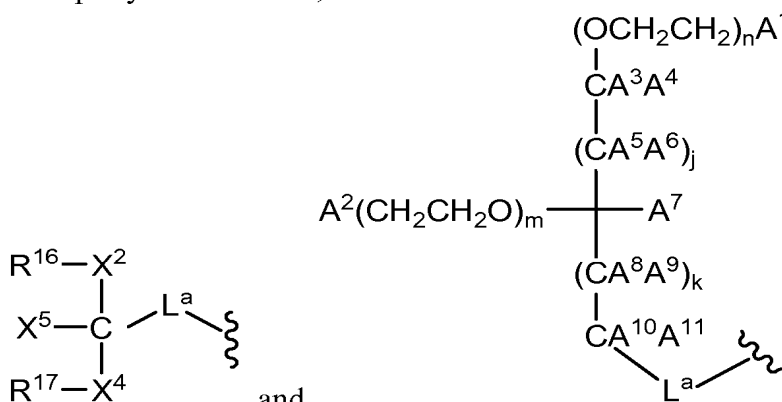
[0297] In another exemplary embodiment, the nucleotide sugar is based upon the following formula:



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in which the R groups, and L, represent moieties as discussed above. The index "y" is 0, 1 or 2. In an exemplary embodiment, L is a bond between NH and  $\text{R}^1$ . The base is a nucleic acid base.

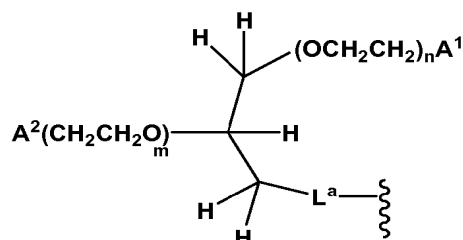
[0298] In an exemplary embodiment,  $\text{L-R}^1$  is a member selected from



10

and  
in which the variables are as described above.

[0299] In an exemplary embodiment,  $\text{L-R}^1$  has a structure according to the following formula:



In an exemplary embodiment,  $A^1$  and  $A^2$  are each selected from  $-OH$  and  $-OCH_3$ .

### III. The Methods

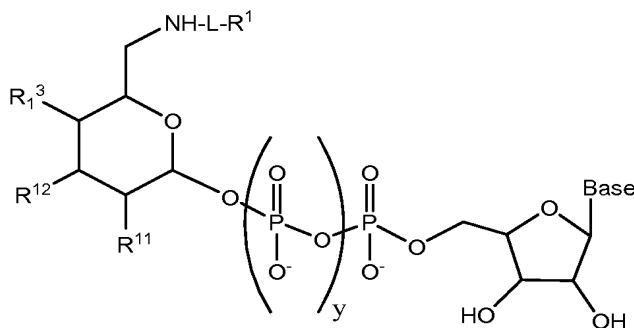
[0300] In addition to the conjugates discussed above, the present invention provides methods for preparing these and other conjugates. Moreover, the invention provides methods of preventing, curing or ameliorating a disease state by administering a conjugate of the invention to a subject at risk of developing the disease or a subject that has the disease.

[0301] In exemplary embodiments, the conjugate is formed between a polymeric modifying moiety and a glycosylated or non-glycosylated peptide. The polymer is conjugated to the peptide via a glycosyl linking group, which is interposed between, and covalently linked to both the peptide (or glycosyl residue) and the modifying group (e.g., water-soluble polymer). The method includes contacting the peptide with a mixture containing a modified sugar and an enzyme, e.g., a glycosyltransferase that conjugates the modified sugar to the substrate. The reaction is conducted under conditions appropriate to form a covalent bond between the modified sugar and the peptide. The sugar moiety of the modified sugar is preferably selected from nucleotide sugars. The method of synthesizing a peptide conjugate, comprising combining a) sialidase; b) an enzyme capable of catalyzing the transfer of a glycosyl linking group such as a glycosyltransferase, exoglycosidase or endoglycosidase; c) modified sugar; d) peptide, thus synthesizing the peptide conjugate. The reaction is conducted under conditions appropriate to form a covalent bond between the modified sugar and the peptide. The sugar moiety of the modified sugar is preferably selected from nucleotide sugars.

[0302] In an exemplary embodiment, the modified sugar, such as those set forth above, is activated as the corresponding nucleotide sugars. Exemplary sugar nucleotides that are used in the present invention in their modified form include nucleotide mono-, di- or triphosphates or analogs thereof. In a preferred embodiment, the modified sugar nucleotide is selected from a UDP-glycoside, CMP-glycoside, or a GDP-glycoside. Even more preferably, the sugar nucleotide portion of the modified sugar nucleotide is selected from UDP-galactose,

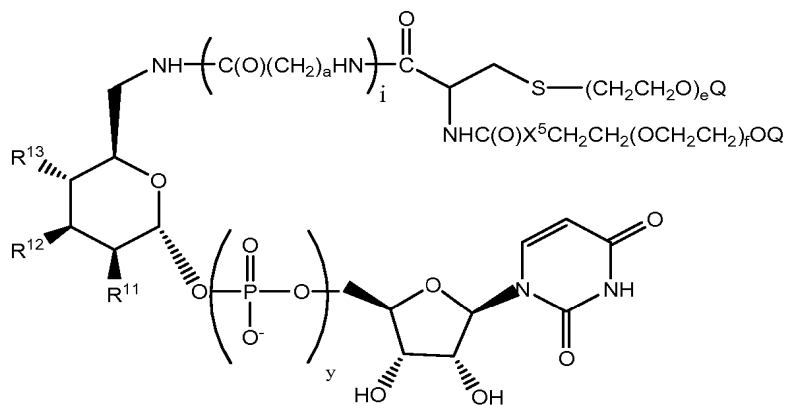
UDP-galactosamine, UDP-glucose, UDP-glucosamine, GDP-mannose, GDP-fucose, CMP-sialic acid, or CMP-NeuAc. In an exemplary embodiment, the nucleotide phosphate is attached to C-1.

- 5 [0303] The invention also provides for the use of sugar nucleotides modified with L-R<sup>1</sup> at the 6-carbon position. Exemplary species according to this embodiment include:



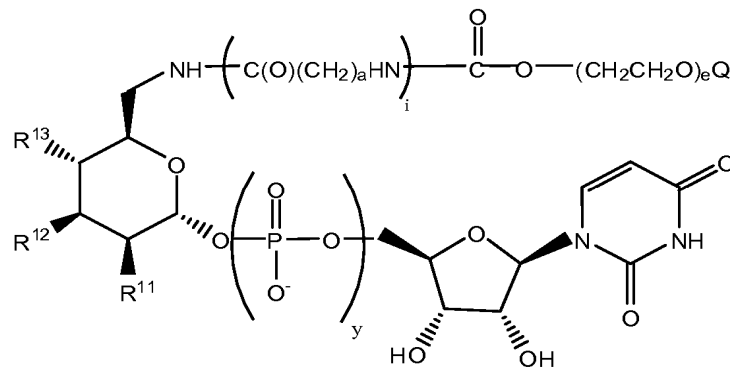
in which the R groups, and L, represent moieties as discussed above. The index “y” is 0, 1 or 2. In an exemplary embodiment, L is a bond between NH and R<sup>1</sup>. The base is a nucleic acid base.

- 10 [0304] Exemplary nucleotide sugars of use in the invention are described herein. In another exemplary embodiment, nucleotide sugars of use in the invention are those in which the carbon at the 6-position is modified include species having the stereochemistry of GDP mannose, e.g.:



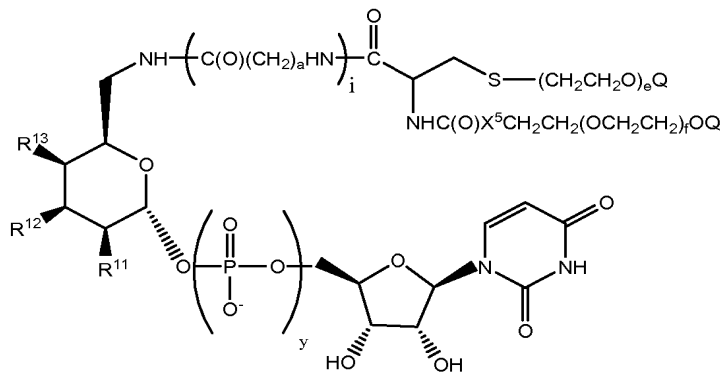
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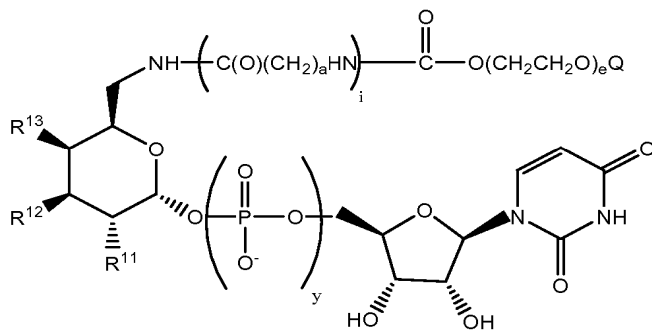


in which  $X^5$  is a bond or O and the remaining variables are as described above. The index  $i$  represents 0 or 1. The index  $a$  represents an integer from 1 to 20. The indices  $e$  and  $f$  independently represent integers from 1 to 2500.  $Q$ , as discussed above, is H or substituted or unsubstituted  $C_1$ - $C_6$  alkyl. As those of skill will appreciate, the serine derivative, in which S is replaced with O also falls within this general motif.

**[0305]** In a still further exemplary embodiment, the invention provides a conjugate in which the modified sugar is based on the stereochemistry of UDP galactose. An exemplary nucleotide sugar of use in this invention has the structure:

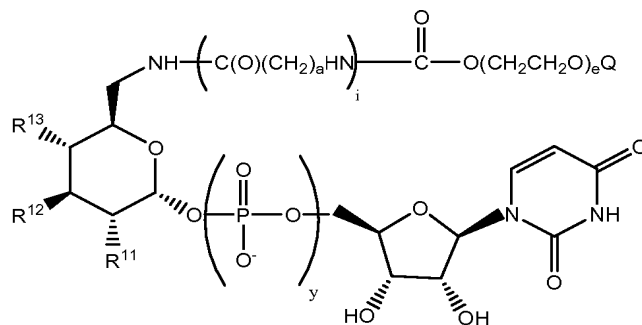
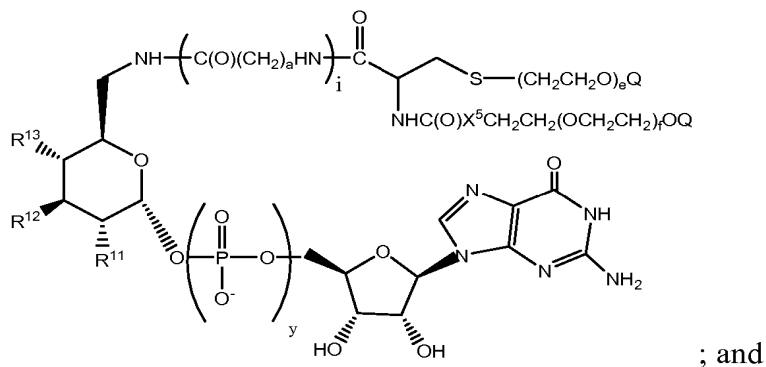


; and



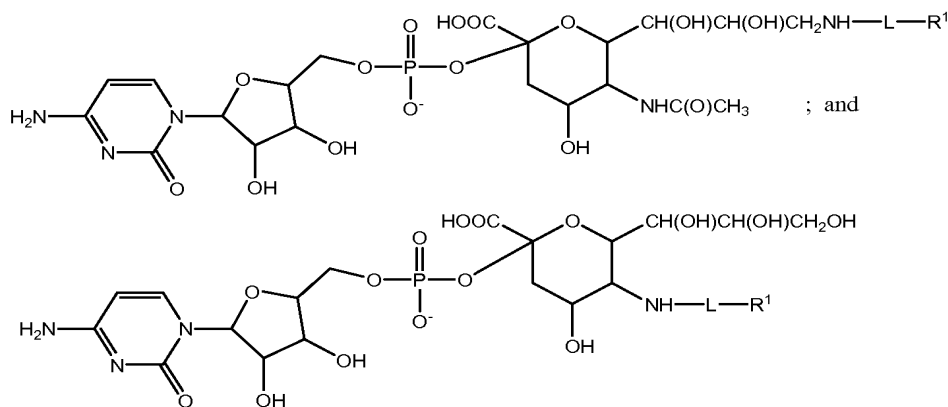
wherein the variables are as described above.

**[0306]** In another exemplary embodiment, the nucleotide sugar is based on the stereochemistry of glucose. Exemplary species according to this embodiment have the formulae:



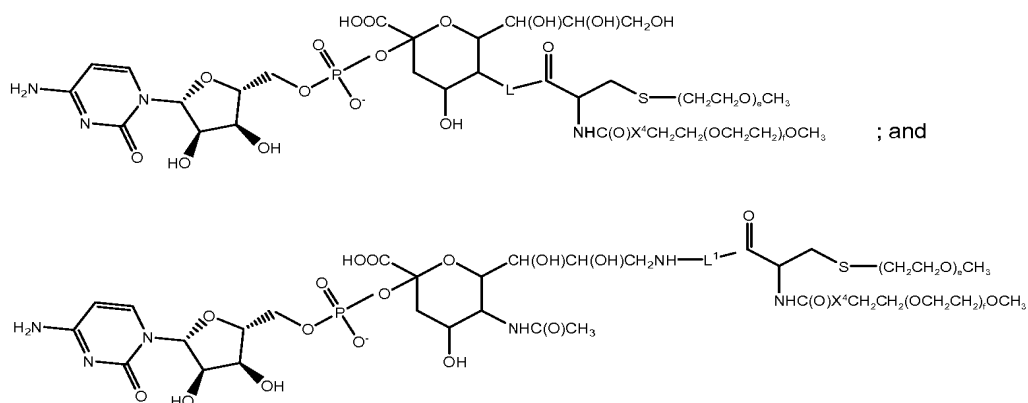
wherein the variables are as described above.

[0307] Thus, in an illustrative embodiment in which the glycosyl moiety is sialic acid, the method of the invention utilizes compounds having the formulae:



in which L-R<sup>1</sup> is as discussed above, and L<sup>1</sup>-R<sup>1</sup> represents a linker bound to the modifying group. As with L, exemplary linker species according to L<sup>1</sup> include a bond, alkyl or heteroalkyl moieties.

[0308] Moreover, as discussed above, the present invention provides for the use of nucleotide sugars that are modified with a water-soluble polymer, which is either straight-chain or branched. For example, compounds having the formula shown below are of use to prepare conjugates within the scope of the present invention:



in which  $X^4$  is O or a bond.

[0309] In general, the sugar moiety or sugar moiety-linker cassette and the PEG or PEG-linker cassette groups are linked together through the use of reactive groups, which are typically transformed by the linking process into a new organic functional group or unreactive species. The sugar reactive functional group(s), is located at any position on the sugar moiety. Reactive groups and classes of reactions useful in practicing the present invention are generally those that are well known in the art of bioconjugate chemistry. Currently favored classes of reactions available with reactive sugar moieties are those, which proceed under relatively mild conditions. These include, but are not limited to nucleophilic substitutions (*e.g.*, reactions of amines and alcohols with acyl halides, active esters), electrophilic substitutions (*e.g.*, enamine reactions) and additions to carbon-carbon and carbon-heteroatom multiple bonds (*e.g.*, Michael reaction, Diels-Alder addition). These and other useful reactions are discussed in, for example, March, *ADVANCED ORGANIC CHEMISTRY*, 3rd Ed., John Wiley & Sons, New York, 1985; Hermanson, *BIOCONJUGATE TECHNIQUES*, Academic Press, San Diego, 1996; and Feeney *et al.*, *MODIFICATION OF PROTEINS*; *Advances in Chemistry Series*, Vol. 198, American Chemical Society, Washington, D.C., 1982.

[0310] Useful reactive functional groups pendent from a sugar nucleus or modifying group include, but are not limited to:

- (a) carboxyl groups and various derivatives thereof including, but not limited to, N-hydroxysuccinimide esters, N-hydroxybenztriazole esters, acid halides, acyl imidazoles, thioesters, p-nitrophenyl esters, alkyl, alkenyl, alkynyl and aromatic esters;
- (b) hydroxyl groups, which can be converted to, *e.g.*, esters, ethers, aldehydes, *etc.*

- (c) haloalkyl groups, wherein the halide can be later displaced with a nucleophilic group such as, for example, an amine, a carboxylate anion, thiol anion, carbanion, or an alkoxide ion, thereby resulting in the covalent attachment of a new group at the functional group of the halogen atom;
- (d) dienophile groups, which are capable of participating in Diels-Alder reactions such as, for example, maleimido groups;
- (e) aldehyde or ketone groups, such that subsequent derivatization is possible via formation of carbonyl derivatives such as, for example, imines, hydrazones, semicarbazones or oximes, or via such mechanisms as Grignard addition or alkyllithium addition;
- (f) sulfonyl halide groups for subsequent reaction with amines, for example, to form sulfonamides;
- (g) thiol groups, which can be, for example, converted to disulfides or reacted with acyl halides;
- (h) amine or sulfhydryl groups, which can be, for example, acylated, alkylated or oxidized;
- (i) alkenes, which can undergo, for example, cycloadditions, acylation, Michael addition, *etc*; and
- (j) epoxides, which can react with, for example, amines and hydroxyl compounds.

[0311] The reactive functional groups can be chosen such that they do not participate in, or interfere with, the reactions necessary to assemble the reactive sugar nucleus or modifying group. Alternatively, a reactive functional group can be protected from participating in the reaction by the presence of a protecting group. Those of skill in the art understand how to protect a particular functional group such that it does not interfere with a chosen set of reaction conditions. For examples of useful protecting groups, *see*, for example, Greene *et al.*, PROTECTIVE GROUPS IN ORGANIC SYNTHESIS, John Wiley & Sons, New York, 1991.

[0312] In the discussion that follows, a number of specific examples of modified sugars that are useful in practicing the present invention are set forth. In the exemplary embodiments, a sialic acid derivative is utilized as the sugar nucleus to which the modifying group is attached. The focus of the discussion on sialic acid derivatives is for clarity of illustration only and should not be construed to limit the scope of the invention. Those of skill in the art will appreciate that a variety of other sugar moieties can be activated and derivatized in a manner analogous to that set forth using sialic acid as an example. For

example, numerous methods are available for modifying galactose, glucose, N-acetylgalactosamine and fucose to name a few sugar substrates, which are readily modified by art recognized methods. *See*, for example, Elhalabi *et al.*, *Curr. Med. Chem.* **6**: 93 (1999); and Schafer *et al.*, *J. Org. Chem.* **65**: 24 (2000)).

5 [0313] In an exemplary embodiment, the modified sugar is based upon a 6-amino-N-acetyl-glycosyl moiety.

[0314] In the scheme above, the index n represents an integer from 1 to 2500. In an exemplary embodiment, this index is selected such that the polymer is about 10 kD, 15 kD or 20 kD in molecular weight. The symbol "A" represents an activating group, e.g., a halo, a  
10 component of an activated ester (e.g., a N-hydroxysuccinimide ester), a component of a carbonate (e.g., p-nitrophenyl carbonate) and the like. Those of skill in the art will appreciate that other PEG-amide nucleotide sugars are readily prepared by this and analogous methods.

[0315] The peptide is typically synthesized *de novo*, or recombinantly expressed in a prokaryotic cell (e.g., bacterial cell, such as *E. coli*) or in a eukaryotic cell such as a  
15 mammalian, yeast, insect, fungal or plant cell. The peptide can be either a full-length protein or a fragment. Moreover, the peptide can be a wild type or mutated peptide. In an exemplary embodiment, the peptide includes a mutation that adds one or more N- or O-linked glycosylation sites to the peptide sequence.

[0316] The method of the invention also provides for modification of incompletely  
20 glycosylated peptides that are produced recombinantly. Many recombinantly produced glycoproteins are incompletely glycosylated, exposing carbohydrate residues that may have undesirable properties, e.g., immunogenicity, recognition by the RES. Employing a modified sugar in a method of the invention, the peptide can be simultaneously further glycosylated and derivatized with, e.g., a water-soluble polymer, therapeutic agent, or the like. The sugar  
25 moiety of the modified sugar can be the residue that would properly be conjugated to the acceptor in a fully glycosylated peptide, or another sugar moiety with desirable properties.

[0317] Those of skill will appreciate that the invention can be practiced using substantially any peptide or glycopeptide from any source. Exemplary peptides with which the invention can be practiced are set forth in WO 03/031464, and the references set forth therein.

30 [0318] Peptides modified by the methods of the invention can be synthetic or wild-type peptides or they can be mutated peptides, produced by methods known in the art, such as site-

directed mutagenesis. Glycosylation of peptides is typically either N-linked or O-linked. An exemplary N-linkage is the attachment of the modified sugar to the side chain of an asparagine residue. The tripeptide sequences asparagine-X-serine and asparagine-X-threonine, where X is any amino acid except proline, are the recognition sequences for enzymatic attachment of a carbohydrate moiety to the asparagine side chain. Thus, the presence of either of these tripeptide sequences in a polypeptide creates a potential glycosylation site. O-linked glycosylation refers to the attachment of one sugar (e.g., N-acetylgalactosamine, galactose, mannose, GlcNAc, glucose, fucose or xylose) to the hydroxy side chain of a hydroxyamino acid, preferably serine or threonine, although unusual or non-natural amino acids, e.g., 5-hydroxyproline or 5-hydroxylysine may also be used.

[0319] Moreover, in addition to peptides, the methods of the present invention can be practiced with other biological structures (e.g., glycolipids, lipids, sphingoids, ceramides, whole cells, and the like, containing a glycosylation site).

[0320] Addition of glycosylation sites to a peptide or other structure is conveniently accomplished by altering the amino acid sequence such that it contains one or more glycosylation sites. The addition may also be made by the incorporation of one or more species presenting an -OH group, preferably serine or threonine residues, within the sequence of the peptide (for O-linked glycosylation sites). The addition may be made by mutation or by full chemical synthesis of the peptide. The peptide amino acid sequence is preferably altered through changes at the DNA level, particularly by mutating the DNA encoding the peptide at preselected bases such that codons are generated that will translate into the desired amino acids. The DNA mutation(s) are preferably made using methods known in the art.

[0321] In an exemplary embodiment, the glycosylation site is added by shuffling polynucleotides. Polynucleotides encoding a candidate peptide can be modulated with DNA shuffling protocols. DNA shuffling is a process of recursive recombination and mutation, performed by random fragmentation of a pool of related genes, followed by reassembly of the fragments by a polymerase chain reaction-like process. See, e.g., Stemmer, *Proc. Natl. Acad. Sci. USA* 91:10747-10751 (1994); Stemmer, *Nature* 370:389-391 (1994); and U.S. Patent Nos. 5,605,793, 5,837,458, 5,830,721 and 5,811,238.

[0322] Exemplary peptides with which the present invention can be practiced, methods of adding or removing glycosylation sites, and adding or removing glycosyl structures or substructures are described in detail in WO03/031464 and related U.S. and PCT applications.

[0323] The present invention also takes advantage of adding to (or removing from) a peptide one or more selected glycosyl residues, after which a modified sugar is conjugated to at least one of the selected glycosyl residues of the peptide. The present embodiment is useful, for example, when it is desired to conjugate the modified sugar to a selected glycosyl residue that is either not present on a peptide or is not present in a desired amount. Thus, prior to coupling a modified sugar to a peptide, the selected glycosyl residue is conjugated to the peptide by enzymatic or chemical coupling. In another embodiment, the glycosylation pattern of a glycopeptide is altered prior to the conjugation of the modified sugar by the removal of a carbohydrate residue from the glycopeptide. *See*, for example WO 98/31826.

[0324] Addition or removal of any carbohydrate moieties present on the glycopeptide is accomplished either chemically or enzymatically. An exemplary chemical deglycosylation is brought about by exposure of the polypeptide variant to the compound trifluoromethanesulfonic acid, or an equivalent compound. This treatment results in the cleavage of most or all sugars except the linking sugar (N-acetylglucosamine or N-acetylgalactosamine), while leaving the peptide intact. Chemical deglycosylation is described by Hakimuddin *et al.*, *Arch. Biochem. Biophys.* **259**: 52 (1987) and by Edge *et al.*, *Anal. Biochem.* **118**: 131 (1981). Enzymatic cleavage of carbohydrate moieties on polypeptide variants can be achieved by the use of a variety of endo- and exo-glycosidases as described by Thotakura *et al.*, *Meth. Enzymol.* **138**: 350 (1987).

[0325] In an exemplary embodiment, the peptide is essentially completely desialylated with neuraminidase prior to performing glycoconjugation or remodeling steps on the peptide. Following the glycoconjugation or remodeling, the peptide is optionally re-sialylated using a sialyltransferase. In an exemplary embodiment, the re-sialylation occurs at essentially each (e.g., >80%, preferably greater than 85%, greater than 90%, preferably greater than 95% and more preferably greater than 96%, 97%, 98% or 99%) terminal saccharyl acceptor in a population of sialyl acceptors. In a preferred embodiment, the saccharide has a substantially uniform sialylation pattern (i.e., substantially uniform glycosylation pattern).

[0326] Chemical addition of glycosyl moieties is carried out by any art-recognized method. Enzymatic addition of sugar moieties is preferably achieved using a modification of the methods set forth herein, substituting native glycosyl units for the modified sugars used in the invention. Other methods of adding sugar moieties are disclosed in U.S. Patent No. 5,876,980, 6,030,815, 5,728,554, and 5,922,577.

[0327] Exemplary attachment points for selected glycosyl residue include, but are not limited to: (a) consensus sites for N-linked glycosylation, and sites for O-linked glycosylation; (b) terminal glycosyl moieties that are acceptors for a glycosyltransferase; (c) arginine, asparagine and histidine; (d) free carboxyl groups; (e) free sulfhydryl groups such as those of cysteine; (f) free hydroxyl groups such as those of serine, threonine, or hydroxyproline; (g) aromatic residues such as those of phenylalanine, tyrosine, or tryptophan; or (h) the amide group of glutamine. Exemplary methods of use in the present invention are described in WO 87/05330 published Sep. 11, 1987, and in Aplin and Wriston, CRC CRIT. REV. BIOCHEM., pp. 259-306 (1981).

[0328] In one embodiment, the invention provides a method for linking two or more peptides through a linking group. The linking group is of any useful structure and may be selected from straight- and branched-chain structures. Preferably, each terminus of the linker, which is attached to a peptide, includes a modified sugar (i.e., a nascent intact glycosyl linking group).

[0329] In an exemplary method of the invention, two peptides are linked together via a linker moiety that includes a polymeric (e.g., PEG linker). The construct conforms to the general structure set forth in the cartoon above. As described herein, the construct of the invention includes two intact glycosyl linking groups (i.e.,  $s + t = 1$ ). The focus on a PEG linker that includes two glycosyl groups is for purposes of clarity and should not be interpreted as limiting the identity of linker arms of use in this embodiment of the invention.

[0330] Thus, a PEG moiety is functionalized at a first terminus with a first glycosyl unit and at a second terminus with a second glycosyl unit. The first and second glycosyl units are preferably substrates for different transferases, allowing orthogonal attachment of the first and second peptides to the first and second glycosyl units, respectively. In practice, the (glycosyl)<sup>1</sup>-PEG-(glycosyl)<sup>2</sup> linker is contacted with the first peptide and a first transferase for which the first glycosyl unit is a substrate, thereby forming (peptide)<sup>1</sup>-(glycosyl)<sup>1</sup>-PEG-(glycosyl)<sup>2</sup>. Transferase and/or unreacted peptide is then optionally removed from the reaction mixture. The second peptide and a second transferase for which the second glycosyl unit is a substrate are added to the (peptide)<sup>1</sup>-(glycosyl)<sup>1</sup>-PEG-(glycosyl)<sup>2</sup> conjugate, forming (peptide)<sup>1</sup>-(glycosyl)<sup>1</sup>-PEG-(glycosyl)<sup>2</sup>-(peptide)<sup>2</sup>; at least one of the glycosyl residues is either directly or indirectly O-linked. Those of skill in the art will appreciate that the method

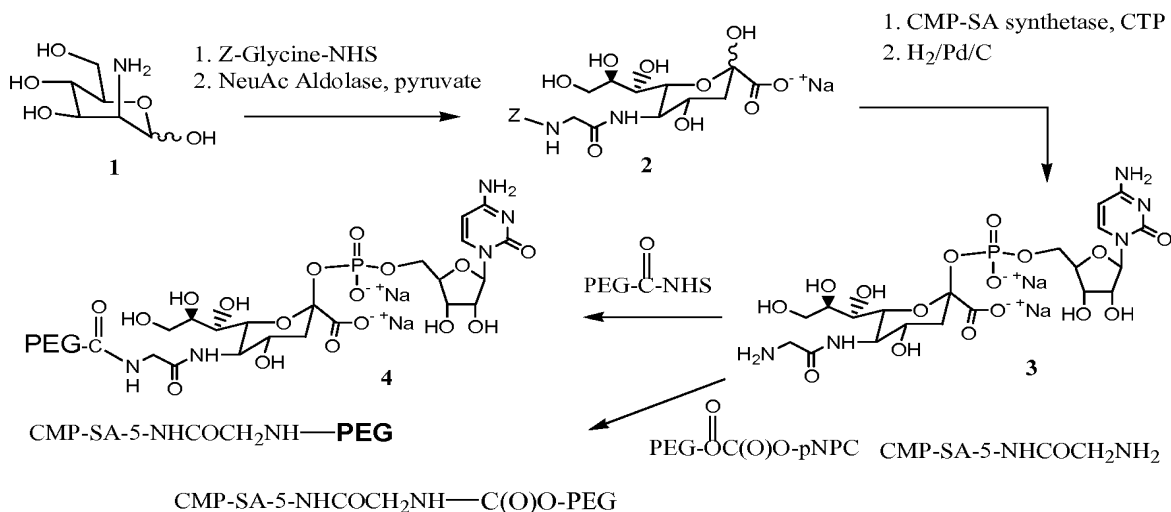


outlined above is also applicable to forming conjugates between more than two peptides by, for example, the use of a branched PEG, dendrimer, poly(amino acid), polysaccharide or the like.

[0331] In an exemplary embodiment, the peptide that is modified by a method of the invention is a glycopeptide that is produced in mammalian cells (e.g., CHO cells) or in a transgenic animal and thus, contains N- and/or O-linked oligosaccharide chains, which are incompletely sialylated. The oligosaccharide chains of the glycopeptide lacking a sialic acid and containing a terminal galactose residue can be PEGylated, PPGylated or otherwise modified with a modified sialic acid.

[0332] In Scheme 1, the amino glycoside **1**, is treated with the active ester of a protected amino acid (e.g., glycine) derivative, converting the sugar amine residue into the corresponding protected amino acid amide adduct. The adduct is treated with an aldolase to form  $\alpha$ -hydroxy carboxylate **2**. Compound **2** is converted to the corresponding CMP derivative by the action of CMP-SA synthetase, followed by catalytic hydrogenation of the CMP derivative to produce compound **3**. The amine introduced via formation of the glycine adduct is utilized as a locus of PEG attachment by reacting compound **3** with an activated PEG or PPG derivative (e.g., PEG-C(O)NHS, PEG-OC(O)O-p-nitrophenyl), producing species such as **4** or **5**, respectively.

**Scheme 1**



In an exemplary embodiment, a modified sugar can be attached to an O-glycan binding site on a peptide. The glycosyltransferases which can be used to produce this peptide conjugate include: for Ser56 (-Glc-(Xyl)<sub>n</sub>-Gal-SA-PEG – a galactosyltransferase and sialyltransferase; for Ser56 –Glc-(Xyl)<sub>n</sub>-Xyl-PEG – a xylosyltransferase; and for Ser60-Fuc-GlcNAc-(Gal)<sub>n</sub>-  
5 (SA)<sub>m</sub>-PEG – a GlcNAc transferase.

### **III. A. Conjugation of Modified Sugars to Peptides**

[0333] The PEG modified sugars are conjugated to a glycosylated or non-glycosylated peptide using an appropriate enzyme to mediate the conjugation. Preferably, the concentrations of the modified donor sugar(s), enzyme(s) and acceptor peptide(s) are selected  
10 such that glycosylation proceeds until the acceptor is consumed. The considerations discussed below, while set forth in the context of a sialyltransferase, are generally applicable to other glycosyltransferase reactions. A list of preferred sialyltransferases for use in the invention is provided in **FIG. 6**.

[0334] A number of methods of using glycosyltransferases to synthesize desired  
15 oligosaccharide structures are known and are generally applicable to the instant invention. Exemplary methods are described, for instance, WO 96/32491, Ito *et al.*, *Pure Appl. Chem.* **65**: 753 (1993), U.S. Pat. Nos. 5,352,670, 5,374,541, 5,545,553, commonly owned U.S. Pat. Nos. 6,399,336, and 6,440,703, and commonly owned published PCT applications, WO 03/031464, WO 04/033651, WO 04/099231, which are incorporated herein by reference.

[0335] The present invention is practiced using a single glycosyltransferase or a combination of glycosyltransferases. For example, one can use a combination of a sialyltransferase and a galactosyltransferase. In those embodiments using more than one enzyme, the enzymes and substrates are preferably combined in an initial reaction mixture, or the enzymes and reagents for a second enzymatic reaction are added to the reaction medium  
20 once the first enzymatic reaction is complete or nearly complete. By conducting two enzymatic reactions in sequence in a single vessel, overall yields are improved over procedures in which an intermediate species is isolated. Moreover, cleanup and disposal of extra solvents and by-products is reduced.

[0336] In a preferred embodiment, each of the first and second enzyme is a  
30 glycosyltransferase. In another preferred embodiment, one enzyme is an endoglycosidase. In an additional preferred embodiment, more than two enzymes are used to assemble the

modified glycoprotein of the invention. The enzymes are used to alter a saccharide structure on the peptide at any point either before or after the addition of the modified sugar to the peptide.

[0337] In another embodiment, the method makes use of one or more exo- or endoglycosidase. The glycosidase is typically a mutant, which is engineered to form glycosyl bonds rather than rupture them. The mutant glycanase typically includes a substitution of an amino acid residue for an active site acidic amino acid residue. For example, when the endoglycanase is endo-H, the substituted active site residues will typically be Asp at position 130, Glu at position 132 or a combination thereof. The amino acids are generally replaced with serine, alanine, asparagine, or glutamine.

[0338] The mutant enzyme catalyzes the reaction, usually by a synthesis step that is analogous to the reverse reaction of the endoglycanase hydrolysis step. In these embodiments, the glycosyl donor molecule (*e.g.*, a desired oligo- or mono-saccharide structure) contains a leaving group and the reaction proceeds with the addition of the donor molecule to a GlcNAc residue on the protein. For example, the leaving group can be a halogen, such as fluoride. In other embodiments, the leaving group is a Asn, or a Asn-peptide moiety. In further embodiments, the GlcNAc residue on the glycosyl donor molecule is modified. For example, the GlcNAc residue may comprise a 1,2 oxazoline moiety.

[0339] In a preferred embodiment, each of the enzymes utilized to produce a conjugate of the invention are present in a catalytic amount. The catalytic amount of a particular enzyme varies according to the concentration of that enzyme's substrate as well as to reaction conditions such as temperature, time and pH value. Means for determining the catalytic amount for a given enzyme under preselected substrate concentrations and reaction conditions are well known to those of skill in the art.

[0340] The temperature at which an above process is carried out can range from just above freezing to the temperature at which the most sensitive enzyme denatures. Preferred temperature ranges are about 0 °C to about 55 °C, and more preferably about 20 °C to about 37 °C. In another exemplary embodiment, one or more components of the present method are conducted at an elevated temperature using a thermophilic enzyme.

[0341] The reaction mixture is maintained for a period of time sufficient for the acceptor to be glycosylated, thereby forming the desired conjugate. Some of the conjugate can often be

detected after a few h, with recoverable amounts usually being obtained within 24 h or less. Those of skill in the art understand that the rate of reaction is dependent on a number of variable factors (*e.g.*, enzyme concentration, donor concentration, acceptor concentration, temperature, solvent volume), which are optimized for a selected system.

5    **[0342]**   The present invention also provides for the industrial-scale production of modified peptides. As used herein, an industrial scale generally produces at least one gram of finished, purified conjugate.

10   **[0343]**   In the discussion that follows, the invention is exemplified by the conjugation of modified sialic acid moieties to a glycosylated peptide. The exemplary modified sialic acid is labeled with PEG. The focus of the following discussion on the use of PEG-modified sialic acid and glycosylated peptides is for clarity of illustration and is not intended to imply that the invention is limited to the conjugation of these two partners. One of skill understands that the discussion is generally applicable to the additions of modified glycosyl moieties other than sialic acid. Moreover, the discussion is equally applicable to the modification of a  
15   glycosyl unit with agents other than PEG including other PEG moieties, therapeutic moieties, and biomolecules.

20   **[0344]**   An enzymatic approach can be used for the selective introduction of PEGylated or PPGylated carbohydrates onto a peptide or glycopeptide. The method utilizes modified sugars containing PEG, PPG, or a masked reactive functional group, and is combined with the appropriate glycosyltransferase or glycosynthase. By selecting the glycosyltransferase that will make the desired carbohydrate linkage and utilizing the modified sugar as the donor substrate, the PEG or PPG can be introduced directly onto the peptide backbone, onto existing sugar residues of a glycopeptide or onto sugar residues that have been added to a peptide.

25   **[0345]**   In an exemplary embodiment, an acceptor for a sialyltransferase is present on the peptide to be modified either as a naturally occurring structure or it is placed there recombinantly, enzymatically or chemically. Suitable acceptors, include, for example, galactosyl acceptors such as Gal $\beta$ 1,4GlcNAc, Gal $\beta$ 1,4GalNAc, Gal $\beta$ 1,3GalNAc, lacto-N-tetraose, Gal $\beta$ 1,3GlcNAc, Gal $\beta$ 1,3Ara, Gal $\beta$ 1,6GlcNAc, Gal $\beta$ 1,4Glc (lactose), and other  
30   acceptors known to those of skill in the art (*see, e.g., Paulson et al., J. Biol. Chem.* **253**: 5617-5624 (1978)). Exemplary sialyltransferases are set forth herein.

[0346] In one embodiment, an acceptor for the sialyltransferase is present on the glycopeptide to be modified upon *in vivo* synthesis of the glycopeptide. Such glycopeptides can be sialylated using the claimed methods without prior modification of the glycosylation pattern of the glycopeptide. Alternatively, the methods of the invention can be used to  
5 sialylate a peptide that does not include a suitable acceptor; one first modifies the peptide to include an acceptor by methods known to those of skill in the art. In an exemplary embodiment, a GalNAc residue is added by the action of a GalNAc transferase.

[0347] In an exemplary embodiment, the galactosyl acceptor is assembled by attaching a galactose residue to an appropriate acceptor linked to the peptide, e.g., a GlcNAc. The  
10 method includes incubating the peptide to be modified with a reaction mixture that contains a suitable amount of a galactosyltransferase (e.g., Gal $\beta$ 1,3 or Gal $\beta$ 1,4), and a suitable galactosyl donor (e.g., UDP-galactose). The reaction is allowed to proceed substantially to completion or, alternatively, the reaction is terminated when a preselected amount of the galactose residue is added. Other methods of assembling a selected saccharide acceptor will  
15 be apparent to those of skill in the art.

[0348] In yet another embodiment, glycopeptide-linked oligosaccharides are first “trimmed,” either in whole or in part, to expose either an acceptor for the sialyltransferase or a moiety to which one or more appropriate residues can be added to obtain a suitable acceptor. Enzymes such as glycosyltransferases and endoglycosidases (*see*, for example U.S.  
20 Patent No. 5,716,812) are useful for the attaching and trimming reactions. In another embodiment of this method, the sialic acid moieties of the peptide are essentially completely removed (e.g., at least 90, at least 95 or at least 99%), exposing an acceptor for a modified sialic acid.

[0349] In the discussion that follows, the method of the invention is exemplified by the use  
25 of modified sugars having a PEG moiety attached thereto. The focus of the discussion is for clarity of illustration. Those of skill will appreciate that the discussion is equally relevant to those embodiments in which the modified sugar bears a therapeutic moiety, biomolecule or the like.

[0350] In an exemplary embodiment of the invention in which a carbohydrate residue is  
30 “trimmed” prior to the addition of the modified sugar high mannose is trimmed back to the first generation biantennary structure. A modified sugar bearing a PEG moiety is conjugated to one or more of the sugar residues exposed by the “trimming back.” In one example, a PEG

moiety is added via a GlcNAc moiety conjugated to the PEG moiety. The modified GlcNAc is attached to one or both of the terminal mannose residues of the biantennary structure. Alternatively, an unmodified GlcNAc can be added to one or both of the termini of the branched species.

5 [0351] In another exemplary embodiment, a PEG moiety is added to one or both of the terminal mannose residues of the biantennary structure via a modified sugar having a galactose residue, which is conjugated to a GlcNAc residue added onto the terminal mannose residues. Alternatively, an unmodified Gal can be added to one or both terminal GlcNAc residues.

10 [0352] In yet a further example, a PEG moiety is added onto a Gal residue using a modified sialic acid such as those discussed above.

[0353] In another exemplary embodiment, a high mannose structure is “trimmed back” to the mannose from which the biantennary structure branches. In one example, a PEG moiety is added via a GlcNAc modified with the polymer. Alternatively, an unmodified GlcNAc is  
15 added to the mannose, followed by a Gal with an attached PEG moiety. In yet another embodiment, unmodified GlcNAc and Gal residues are sequentially added to the mannose, followed by a sialic acid moiety modified with a PEG moiety.

[0354] A high mannose structure can also be trimmed back to the elementary tri-mannosyl core.

20 [0355] In a further exemplary embodiment, high mannose is “trimmed back” to the GlcNAc to which the first mannose is attached. The GlcNAc is conjugated to a Gal residue bearing a PEG moiety. Alternatively, an unmodified Gal is added to the GlcNAc, followed by the addition of a sialic acid modified with a water-soluble sugar. In yet a further example, the terminal GlcNAc is conjugated with Gal and the GlcNAc is subsequently fucosylated  
25 with a modified fucose bearing a PEG moiety.

[0356] High mannose may also be trimmed back to the first GlcNAc attached to the Asn of the peptide. In one example, the GlcNAc of the GlcNAc-(Fuc)<sub>a</sub> residue is conjugated with a GlcNAc bearing a water soluble polymer. In another example, the GlcNAc of the GlcNAc-(Fuc)<sub>a</sub> residue is modified with Gal, which bears a water soluble polymer. In a still  
30 further embodiment, the GlcNAc is modified with Gal, followed by conjugation to the Gal of a sialic acid modified with a PEG moiety.

[0357] Other exemplary embodiments are set forth in commonly owned U.S. Patent application Publications: 20040132640; 20040063911; 20040137557; U.S. Patent application Nos: 10/369,979; 10/410,913; 10/360,770; 10/410,945 and PCT/US02/32263 each of which is incorporated herein by reference.

5 [0358] The Examples set forth above provide an illustration of the power of the methods set forth herein. Using the methods described herein, it is possible to “trim back” and build up a carbohydrate residue of substantially any desired structure. The modified sugar can be added to the termini of the carbohydrate moiety as set forth above, or it can be intermediate between the peptide core and the terminus of the carbohydrate.

10 [0359] In an exemplary embodiment, an existing sialic acid is removed from a glycopeptide using a sialidase, thereby unmasking all or most of the underlying galactosyl residues. Alternatively, a peptide or glycopeptide is labeled with galactose residues, or an oligosaccharide residue that terminates in a galactose unit. Following the exposure of or addition of the galactose residues, an appropriate sialyltransferase is used to add a modified  
15 sialic acid.

[0360] In another exemplary embodiment, an enzyme that transfers sialic acid onto sialic acid is utilized. This method can be practiced without treating a sialylated glycan with a sialidase to expose glycan residues beneath the sialic acid. An exemplary polymer-modified sialic acid is a sialic acid modified with poly(ethylene glycol). Other exemplary enzymes  
20 that add sialic acid and modified sialic acid moieties onto glycans that include a sialic acid residue or exchange an existing sialic acid residue on a glycan for these species include ST3Gal3, CST-II, ST8Sia-II, ST8Sia-III and ST8Sia-IV.

[0361] In yet a further approach, a masked reactive functionality is present on the sialic acid. The masked reactive group is preferably unaffected by the conditions used to attach the  
25 modified sialic acid to the Factor VII/Factor VIIa peptide. After the covalent attachment of the modified sialic acid to the peptide, the mask is removed and the peptide is conjugated with an agent such as PEG. The agent is conjugated to the peptide in a specific manner by its reaction with the unmasked reactive group on the modified sugar residue.

[0362] Any modified sugar can be used with its appropriate glycosyltransferase, depending  
30 on the terminal sugars of the oligosaccharide side chains of the glycopeptide. As discussed above, the terminal sugar of the glycopeptide required for introduction of the PEGylated

structure can be introduced naturally during expression or it can be produced post expression using the appropriate glycosidase(s), glycosyltransferase(s) or mix of glycosidase(s) and glycosyltransferase(s).

[0363] In a further exemplary embodiment, UDP-galactose-PEG is reacted with  $\beta$ 1,4-galactosyltransferase, thereby transferring the modified galactose to the appropriate terminal N-acetylglucosamine structure. The terminal GlcNAc residues on the glycopeptide may be produced during expression, as may occur in such expression systems as mammalian, insect, plant or fungus, but also can be produced by treating the glycopeptide with a sialidase and/or glycosidase and/or glycosyltransferase, as required.

[0364] In another exemplary embodiment, a GlcNAc transferase, such as GNT1-5, is utilized to transfer PEGylated-GlcNAc to a terminal mannose residue on a glycopeptide. In a still further exemplary embodiment, the N- and/or O-linked glycan structures are enzymatically removed from a glycopeptide to expose an amino acid or a terminal glycosyl residue that is subsequently conjugated with the modified sugar. For example, an endoglycanase is used to remove the N-linked structures of a glycopeptide to expose a terminal GlcNAc as a GlcNAc-linked-Asn on the glycopeptide. UDP-Gal-PEG and the appropriate galactosyltransferase is used to introduce the PEG-galactose functionality onto the exposed GlcNAc.

[0365] In an alternative embodiment, the modified sugar is added directly to the peptide backbone using a glycosyltransferase known to transfer sugar residues to the peptide backbone. Exemplary glycosyltransferases useful in practicing the present invention include, but are not limited to, GalNAc transferases (GalNAc T1-14), GlcNAc transferases, fucosyltransferases, glucosyltransferases, xylosyltransferases, mannosyltransferases and the like. Use of this approach allows the direct addition of modified sugars onto peptides that lack any carbohydrates or, alternatively, onto existing glycopeptides. In both cases, the addition of the modified sugar occurs at specific positions on the peptide backbone as defined by the substrate specificity of the glycosyltransferase and not in a random manner as occurs during modification of a protein's peptide backbone using chemical methods. An array of agents can be introduced into proteins or glycopeptides that lack the glycosyltransferase substrate peptide sequence by engineering the appropriate amino acid sequence into the polypeptide chain.



[0366] In each of the exemplary embodiments set forth above, one or more additional chemical or enzymatic modification steps can be utilized following the conjugation of the modified sugar to the peptide. In an exemplary embodiment, an enzyme (*e.g.*, fucosyltransferase) is used to append a glycosyl unit (*e.g.*, fucose) onto the terminal modified sugar attached to the peptide. In another example, an enzymatic reaction is utilized to “cap” sites to which the modified sugar failed to conjugate. Alternatively, a chemical reaction is utilized to alter the structure of the conjugated modified sugar. For example, the conjugated modified sugar is reacted with agents that stabilize or destabilize its linkage with the peptide component to which the modified sugar is attached. In another example, a component of the modified sugar is deprotected following its conjugation to the peptide. One of skill will appreciate that there is an array of enzymatic and chemical procedures that are useful in the methods of the invention at a stage after the modified sugar is conjugated to the peptide. Further elaboration of the modified sugar-peptide conjugate is within the scope of the invention.

[0367] Enzymes and reaction conditions for preparing the conjugates of the present invention are discussed in detail in the parent of the instant application as well as co-owned published PCT patent applications WO 03/031464, WO 04/033651, WO 04/099231.

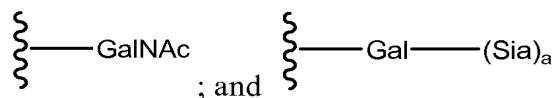
[0368] In a selected embodiment, a peptide, expressed in insect cells, is remodeled such that glycans on the remodeled glycopeptide include a GlcNAc-Gal glycosyl residue. The addition of GlcNAc and Gal can occur as separate reactions or as a single reaction in a single vessel. In this example, GlcNAc-transferase I and Gal-transferase I are used. The modified sialyl moiety is added using ST3Gal-III.

[0369] In another embodiment, the addition of GlcNAc, Gal and modified Sia can also occur in a single reaction vessel, using the enzymes set forth above. Each of the enzymatic remodeling and glycoPEGylation steps are carried out individually.

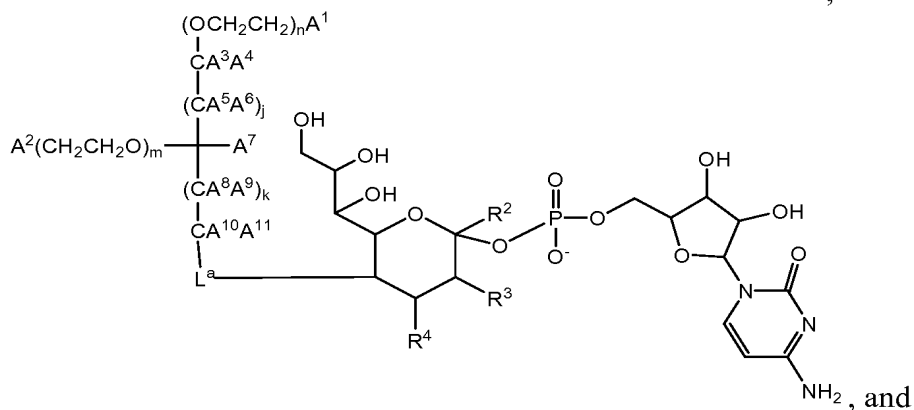
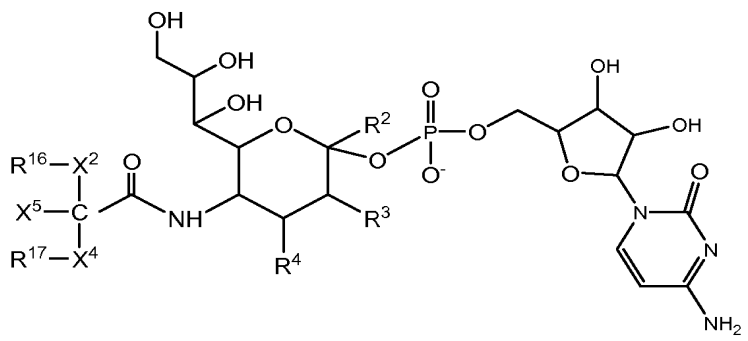
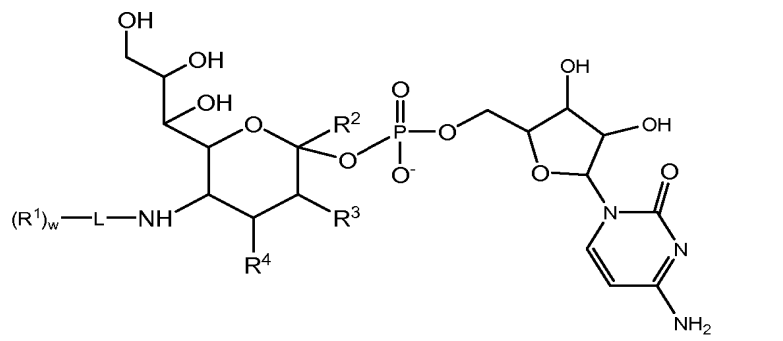
[0370] When the peptide is expressed in mammalian cells, different methods are of use. In one embodiment, the peptide is conjugated without need for remodeling prior to conjugation by contacting the peptide with a sialyltransferase that transfers the modified sialic acid directly onto a sialic acid on the peptide forming Sia-Sia-L-R<sup>1</sup>, or exchanges a sialic acid on the peptide for the modified sialic acid, forming Sia-L-R<sup>1</sup>. An exemplary enzyme of use in this method is CST-II. Other enzymes that add sialic acid to sialic acid are known to those of skill in the art and examples of such enzymes are set forth the figures appended hereto.

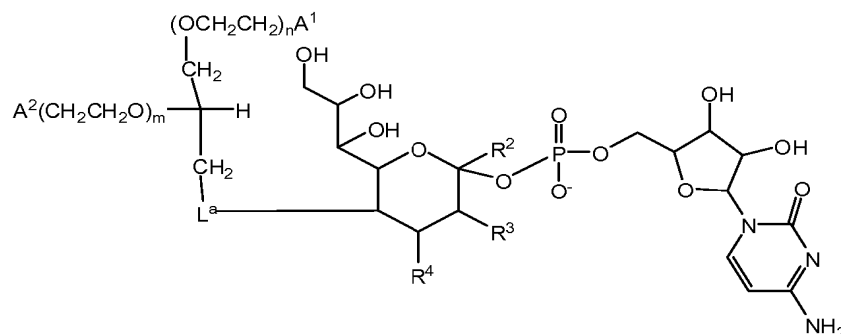
[0371] In yet another method of preparing the conjugates of the invention, the peptide expressed in a mammalian system is desialylated using a sialidase. The exposed Gal residue is sialylated with a modified sialic acid using a sialyltransferase specific for O-linked glycans, providing a peptide with an O-linked modified glycan. The desialylated, modified peptide is optionally partially or fully re-sialylated by using a sialyltransferase such as ST3GalIII.

[0372] In another aspect, the invention provides a method of making a PEGylated peptide conjugate of the invention. The method includes: (a) contacting a peptide comprising a glycosyl group selected from:



10 with a PEG-sialic acid donor having the formula which is a member selected from

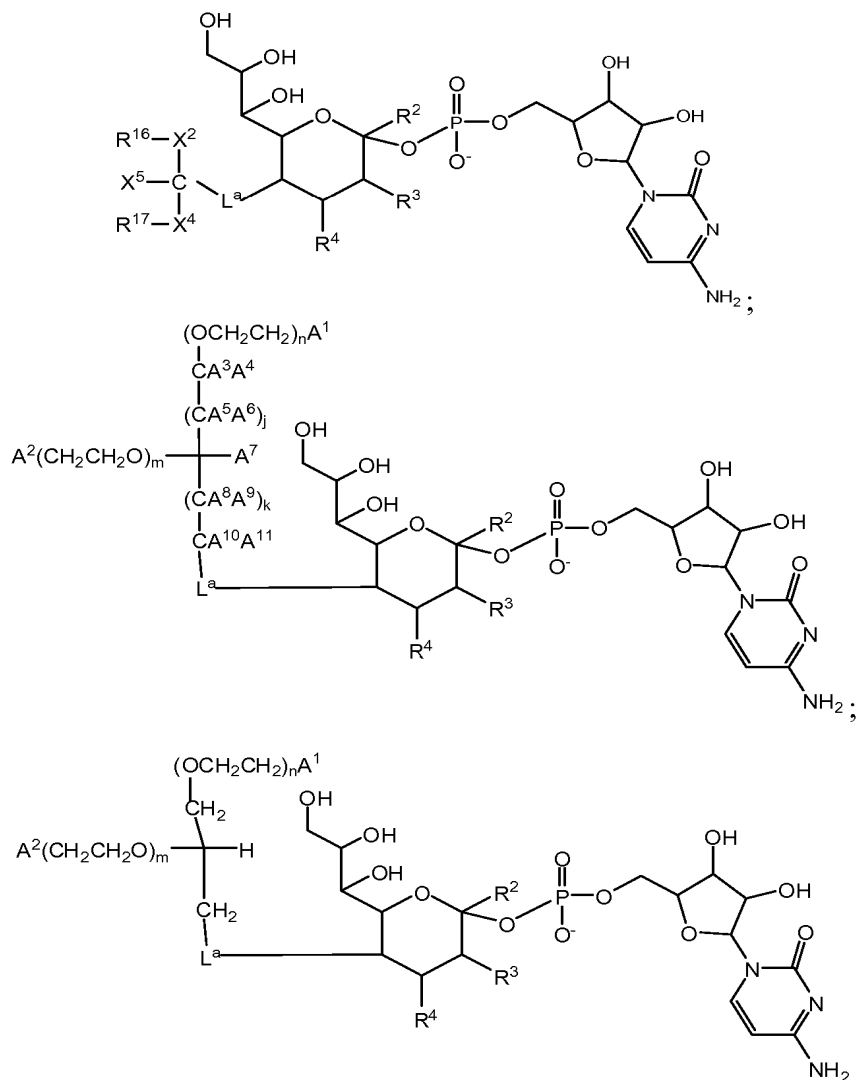




wherein the variables are as described above, and an enzyme that transfers PEG-sialic acid from said donor onto a member selected from the GalNAc, Gal and the Sia of said glycosyl group, under conditions appropriate for said transfer. An exemplary modified sialic acid donor is CMP-sialic acid modified, through a linker moiety, with a polymer, e.g., a straight chain or branched poly(ethylene glycol) moiety. As discussed herein, the peptide is optionally glycosylated with GalNAc and/or Gal and/or Sia (“Remodeled”) prior to attaching the modified sugar. The remodeling steps can occur in sequence in the same vessel without purification of the glycosylated peptide between steps. Alternatively, following one or more remodeling step, the glycosylated peptide can be purified prior to submitting it to the next glycosylation or glycPEGylation step. In an exemplary embodiment, the method further comprises expressing the peptide in a host. In an exemplary embodiment, the host is a mammalian cell or an insect cell. In another exemplary embodiment, the mammalian cell is a member selected from a BHK cell and a CHO cell and the insect cell is a *Spodoptera frugiperda* cell.

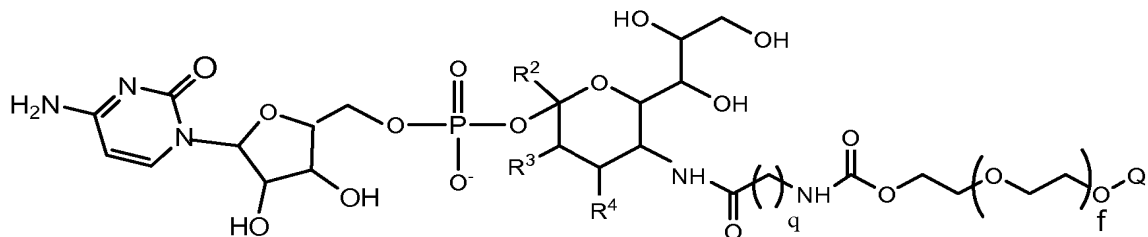
[0373] As illustrated in the examples and discussed further below, placement of an acceptor moiety for the PEG-sugar is accomplished in any desired number of steps. For example, in one embodiment, the addition of GalNAc to the peptide can be followed by a second step in which the PEG-sugar is conjugated to the GalNAc in the same reaction vessel. Alternatively, these two steps can be carried out in a single vessel approximately simultaneously.

[0374] In an exemplary embodiment, the PEG-sialic acid donor has the formula:



wherein the variables are as described above.

- 5 [0375] In another exemplary emodiment, the PEG-sialic acid donor has the formula:

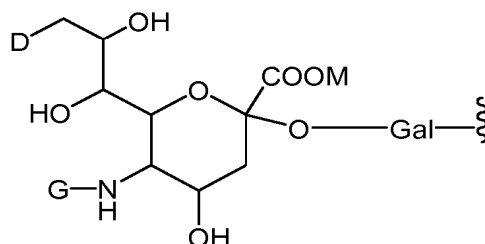


wherein the variables are as described above.

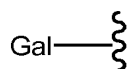
- [0376] In a further exemplary embodiment, the peptide is expressed in an appropriate expression system prior to being glycopeptidated or remodeled. Exemplary expression systems include Sf-9/baculovirus and Chinese Hamster Ovary (CHO) cells.

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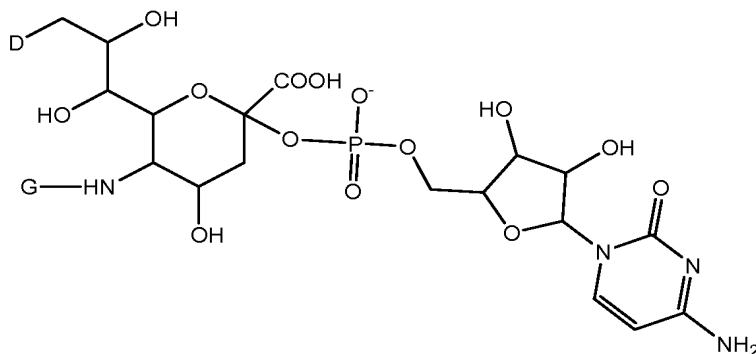
[0377] In an exemplary embodiment, the invention provides a method of making a peptide conjugate comprising a glycosyl linker comprising a modified sialyl residue having the formula:



- 5 wherein D is a member selected from -OH and  $R^1$ -L-NH-; G is a member selected from  $R^1$ -L- and  $-C(O)(C_1-C_6)alkyl-R^1$ ;  $R^1$  is a moiety comprising a member selected from a straight-chain poly(ethylene glycol) residue and branched poly(ethylene glycol) residue; M is a member selected from H, a metal and a single negative charge; L is a linker which is a member selected from a bond, substituted or unsubstituted alkyl and substituted or
- 10 unsubstituted heteroalkyl, such that when D is OH, G is  $R^1$ -L-, and when G is  $-C(O)(C_1-C_6)alkyl$ , D is  $R^1$ -L-NH-  
said method comprising: (a) contacting a peptide comprising the glycosyl moiety:

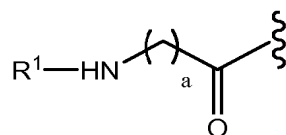


with a PEG-sialic acid donor moiety having the formula:



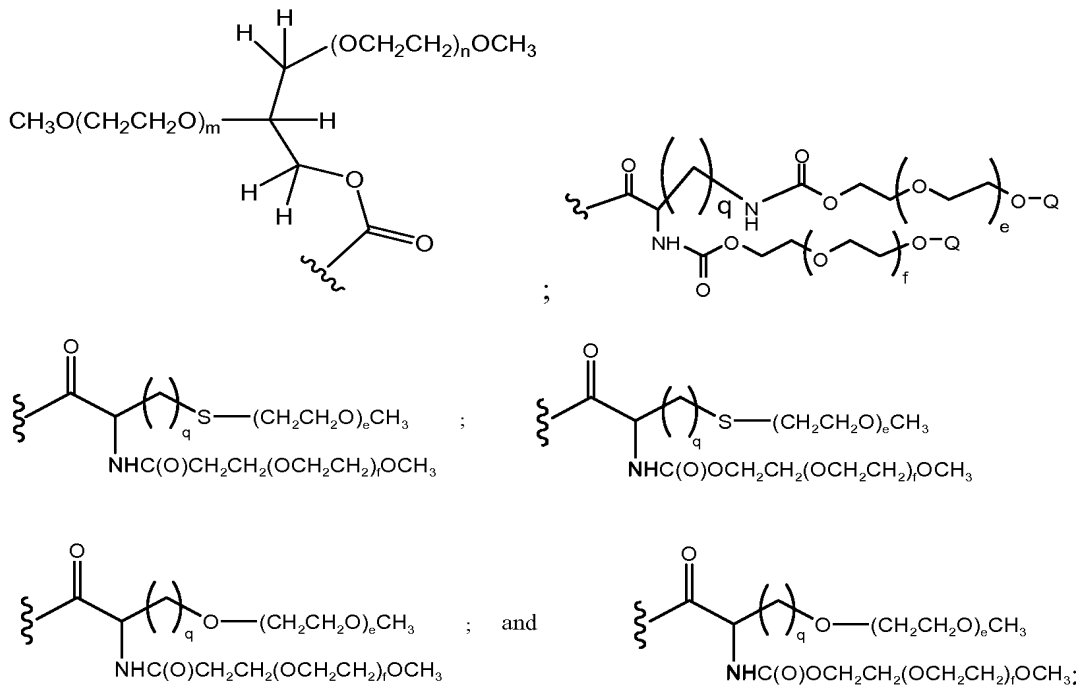
- 15 wherein the variables are as described above, and an enzyme that transfers said PEG-sialic acid onto the Gal of said glycosyl moiety, under conditions appropriate for said transfer.

[0378] In an exemplary embodiment,  $L-R^1$  has the formula:



wherein a is an integer selected from 0 to 20.

[0379] In another exemplary embodiment,  $R^1$  has a structure that is a member selected from:



wherein e, f, m and n are integers independently selected from 1 to 2500; and q is an integer selected from 0 to 20.

[0380] Large scale or small scale amounts of peptide conjugate can be produced by the methods described herein. In an exemplary embodiment, the amount of peptide is a member selected from about 0.5 mg to about 100kg. In an exemplary embodiment, the amount of peptide is a member selected from about 0.1 kg to about 1 kg. In an exemplary embodiment, the amount of peptide is a member selected from about 0.5 kg to about 10kg. In an exemplary embodiment, the amount of peptide is a member selected from about 0.5 kg to about 3kg. In an exemplary embodiment, the amount of peptide is a member selected from about 0.1 kg to about 5kg. In an exemplary embodiment, the amount of peptide is a member selected from about 0.08 kg to about 0.2 kg. In an exemplary embodiment, the amount of peptide is a member selected from about 0.05 kg to about 0.4kg. In an exemplary embodiment, the amount of peptide is a member selected from about 0.1 kg to about 0.7kg. In an exemplary embodiment, the amount of peptide is a member selected from about 0.3 kg

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to about 1.75 kg. In an exemplary embodiment, the amount of peptide is a member selected from about 25 kg to about 65kg.

[0381] The concentration of peptide utilized in the reactions described herein is a member selected from about 0.5 to about 10 mg peptide/mL reaction mixture. In an exemplary  
5 embodiment, the peptide concentration is a member selected from about 0.5 to about 1 mg peptide/mL reaction mixture. In an exemplary embodiment, the peptide concentration is a member selected from about 0.8 to about 3 mg peptide/mL reaction mixture. In an exemplary embodiment, the peptide concentration is a member selected from about 2 to about 6 mg peptide/mL reaction mixture. In an exemplary embodiment, the peptide concentration  
10 is a member selected from about 4 to about 9 mg peptide/mL reaction mixture. In an exemplary embodiment, the peptide concentration is a member selected from about 1.2 to about 7.8 mg peptide/mL reaction mixture. In an exemplary embodiment, the peptide concentration is a member selected from about 6 to about 9.5 mg peptide/mL reaction mixture.

[0382] The concentration of PEGylated nucleotide sugar that can be utilized in the reactions described herein is a member selected from about 0.1 to about 1.0 mM. Factors which may increase or decrease the concentration include the size of the PEG, time of incubation, temperature, buffer components, as well as the type, and concentration, of glycosyltransferase used. In an exemplary embodiment, the PEGylated nucleotide sugar  
20 concentration is a member selected from about 0.1 to about 1.0 mM. In an exemplary embodiment, the PEGylated nucleotide concentration is a member selected from about 0.1 to about 0.5 mM. In an exemplary embodiment, the PEGylated nucleotide sugar concentration is a member selected from about 0.1 to about 0.3 mM. In an exemplary embodiment, the PEGylated nucleotide sugar concentration is a member selected from about 0.2 to about 0.7  
25 mM. In an exemplary embodiment, the PEGylated nucleotide sugar concentration is a member selected from about 0.3 to about 0.5 mM. In an exemplary embodiment, the PEGylated nucleotide sugar concentration is a member selected from about 0.4 to about 1.0 mM. In an exemplary embodiment, the PEGylated nucleotide sugar concentration is a member selected from about 0.5 to about 0.7 mM. In an exemplary embodiment, the  
30 PEGylated nucleotide sugar concentration is a member selected from about 0.8 to about 0.95 mM. In an exemplary embodiment, the PEGylated nucleotide sugar concentration is a member selected from about 0.55 to about 1.0 mM.

[0383] The molar equivalents of the PEGylated nucleotide sugar that can be utilized in the reactions described herein are based on the theoretical number of PEGylated sugars that can be added to the protein. The theoretical number of PEGylated sugars is based on the theoretical number of sugar sites on the protein as well as the MW of the protein when compared to the MW and therefore moles of PEGylated nucleotide sugar. In an exemplary embodiment, the molar equivalents of PEGylated nucleotide sugar is an integer selected from 1 to 20. In an exemplary embodiment, the molar equivalents of PEGylated nucleotide sugar is an integer selected from 1 to 20. In an exemplary embodiment, the molar equivalents of PEGylated nucleotide sugar is an integer selected from 2 to 6. In an exemplary embodiment, the molar equivalents of PEGylated nucleotide sugar is an integer selected from 3 to 17. In an exemplary embodiment, the molar equivalents of PEGylated nucleotide sugar is an integer selected from 4 to 11. In an exemplary embodiment, the molar equivalents of PEGylated nucleotide sugar is an integer selected from 5 to 20. In an exemplary embodiment, the molar equivalents of PEGylated nucleotide sugar is an integer selected from 1 to 10. In an exemplary embodiment, the molar equivalents of PEGylated nucleotide sugar is an integer selected from 12 to 20. In an exemplary embodiment, the molar equivalents of PEGylated nucleotide sugar is an integer selected from 14 to 17. In an exemplary embodiment, the molar equivalents of PEGylated nucleotide sugar is an integer selected from 7 to 15. In an exemplary embodiment, the molar equivalents of PEGylated nucleotide sugar is an integer selected from 8 to 16.

### **III. B. Simultaneous Desialylation and GlycoPEGylation**

[0384] The present invention provides a “one-pot” method of glycopegylating. The one-pot method is distinct from other exemplary processes to make a peptide conjugate, which employ a sequential de-sialylation with sialidase, subsequent purification of the asialopeptide on an anion exchange column, then glycoPEGylation using CMP-sialic acid-PEG and a glycosyltransferase (such as ST3Gal3), exoglycosidase or an endoglycosidase. The peptide conjugate is then purified via anion exchange followed by size exclusion chromatography to produce the purified peptide conjugate.

[0385] The one-pot method is an improved method to manufacture a peptide conjugate. In this method, the de-sialylation and glycoPEGylation reactions are combined in a one-pot reaction which obviates the first anion exchange chromatography step used in the previously described process to purify the asialopeptide. This reduction in process steps produces several advantages. First, the number of process steps required to produce the peptide conjugate is reduced, which



also reduces the operating complexity of the process. Second, the process time for the production of the peptide conjugates is reduced e.g., from 4 to 2 days. This reduces the raw material requirements and quality control costs associated with in-process controls. Third, the invention utilizes less sialidase, e.g., up to 20-fold less sialidase, e.g., 500 mU/L is required to produce the peptide conjugate relative to the process. This reduction in the use of sialidase significantly reduces the amount of contaminants, such as sialidase, in the reaction mixture.

[0386] In an exemplary embodiment, a peptide conjugate is prepared by the following method. In a first step, a peptide is combined with a sialidase, a modified sugar of the invention, and an enzyme capable of catalyzing the transfer of the glycosyl linking group from the modified sugar to the peptide, thus preparing the peptide conjugate. Any sialidase may be used in this method. Exemplary sialidases of use in the invention can be found in the CAZY database (*see* [afmb.cnrs-mrs.fr/CAZY/index.html](http://afmb.cnrs-mrs.fr/CAZY/index.html) and [www.cazy.org/CAZY](http://www.cazy.org/CAZY)).

Exemplary sialidases can be purchased from any number of sources (QA-Bio, Calbiochem, Marukin, Prozyme, etc.). In an exemplary embodiment, the sialidase is a member selected from cytoplasmic sialidases, lysosomal sialidases, exo- $\alpha$  sialidases, and endosialidases. In another exemplary embodiment, the sialidase used is produced from bacteria such as *Clostridium perfringens* or *Streptococcus pneumoniae*, or from a virus such as an adenovirus. In an exemplary embodiment, the enzyme capable of catalyzing the transfer of the glycosyl linking group from the modified sugar to the peptide is a member selected from a glycosyltransferase, such as sialyltransferases and fucosyltransferases, as well as exoglycosidases and endoglycosidases. In an exemplary embodiment, the enzyme is a glycosyltransferase, which is ST3Gal3. In another exemplary embodiment, the enzyme used is produced from bacteria such as *Escherichia Coli* or a fungus such as *Aspergillus niger*. In another exemplary embodiment, the sialidase is added to the peptide before the glycosyltransferase for a specified time, allowing the sialidase reaction to proceed before initiating the GlycoPEGylation reaction with addition of the PEG-sialic acid reagent and the glycosyltransferase. Many of these examples are discussed herein. Finally, any modified sugar described herein can be utilized in this reaction.

[0387] In another exemplary embodiment, the method further comprises a 'capping' step. In this step, additional non-PEGylated sialic acid is added to the reaction mixture. In an exemplary embodiment, this sialic acid is added to the peptide or peptide conjugate thus preventing further addition of PEG-sialic acid. In another exemplary embodiment, this sialic acid impedes the function of the glycosyltransferase in the reaction mixture, effectively

stopping the addition of glycosyl linking groups to the peptides or peptide conjugates. Most importantly, the sialic acid that is added to the reaction mixture caps the unglycoPEGylated glycans thereby providing a peptide conjugate that has improved pharmacokinetics. In addition, this sialidase can be added directly to the glycoPEGylation reaction mixture when the extent of PEGylation to certain amounts is desired without prior purification.

[0388] In an exemplary embodiment, after the capping step, less than about 50% of the sialylation sites on the peptide or peptide conjugate does not comprise a sialyl moiety. In an exemplary embodiment, after the capping step, less than about 40% of the sialylation sites on the peptide or peptide conjugate does not comprise a sialyl moiety. In an exemplary embodiment, after the capping step, less than about 30% of the sialylation sites on the peptide or peptide conjugate does not comprise a sialyl moiety. In an exemplary embodiment, after the capping step, less than about 20% of the sialylation sites on the peptide or peptide conjugate does not comprise a sialyl moiety. In an exemplary embodiment, after the capping step, less than about 10% of the sialylation sites on the peptide or peptide conjugate does not comprise a sialyl moiety. In an exemplary embodiment, between about 20% and about 5% of the sialylation sites on the peptide or peptide conjugate does not comprise a sialyl moiety. In an exemplary embodiment, between about 25% and about 10% of the sialylation sites on the peptide or peptide conjugate does not comprise a sialyl moiety. In an exemplary embodiment, after the capping step, essentially all of the sialylation sites on the peptide or peptide conjugate comprise a sialyl moiety.

### **III. C. Desialylation and Selective Modification of Peptides**

[0389] In another exemplary embodiment, the present invention provides a method for desialylating a peptide. The method preferably provides a peptide that is at least about 40%, preferably 45%, preferably about 50%, preferably about 55%, preferably about 60%, preferably about 65%, preferably about 70%, preferably about 75%, preferably about 80%, preferably at least 85%, more preferably at least 90%, still more preferably, at least 92%, preferably at least 94%, even more preferably at least 96%, still more preferably at least 98%, and still more preferably 100% disialylated.

[0390] The method includes contacting the peptide with a sialidase, preferably for a time period. The preselected time period is sufficient to desialylate the peptide to the degree desired. In a preferred embodiment, the desialylated peptide is separated from the sialidase

when the desired degree of desialylation is achieved. An exemplary desialylation reaction and purification cycle is set forth herein.

[0391] Those of skill are able to determine an appropriate preselected time period over which to conduct the desialylation reaction. In an exemplary embodiment, the period is less than 24 hours, preferably less than 8 hours, more preferably less than 6 hours, more preferably less than 4 hours, still more preferably less than 2 hours and even more preferably less than 1 hour.

[0392] In another exemplary embodiment, in the peptide conjugate preparation at the end of the desialylation reaction, at least 10% of the members of the population of peptides has only a single sialic acid attached thereto, preferably at least 20%, more preferably at least 30%, still more preferably at least 40%, even still more preferably at least 50% and more preferably at least 60%, and still more preferably completely desialylated.

[0393] In yet a further exemplary embodiment, in the preparation at the end of the desialylation reaction, at least 10% of the members of the population of peptides is fully desialylated, preferably at least 20%, more preferably at least 30%, even more preferably at least 40%, still more preferably at least 50% and even still more preferably at least 60%.

[0394] In still another exemplary embodiment, in the preparation at the end of the desialylation reaction, at least 10%, 20%, 30%, 40%, 50% or 60% of the members of the peptide population has only a single sialic acid, and at least 10%, 20%, 30%, 40%, 50% or 60% of the peptide is fully desialylated.

[0395] In a preferred embodiment, in the preparation at the end of the desialylation reaction, at least 50% of the population of peptides is fully desialylated and at least 40% of the members of the peptide population bears only a single sialic acid moiety.

[0396] Following desialylation, the peptide is optionally conjugated with a modified sugar.

An exemplary modified sugar includes a saccharyl moiety bound to a branched or linear poly(ethylene glycol) moiety. The conjugation is catalyzed by an enzyme that transfers the modified sugar from a modified sugar donor onto an amino acid or glycosyl residue of the peptide. An exemplary modified sugar donor is a CMP-sialic acid that bears a branched or linear poly(ethylene glycol) moiety. An exemplary poly(ethylene glycol) moiety has a molecular weight of at least about 2 kD, more preferably at least about 5 kD, more preferably

at least about 10 kD, preferably at least about 20 kD, more preferably at least about 30 kD, and more preferably at least about 40 kD.

[0397] In an exemplary embodiment, the enzyme utilized to transfer the modified sugar moiety from the modified sugar donor is a glycosyltransferase, e.g., sialyltransferase. An  
5 exemplary sialyltransferase of use in the methods of the invention is ST3Gal3.

[0398] An exemplary method of the invention results in a modified peptide bearing at least one, preferably at least two, preferably at least three modifying groups. In one embodiment, the peptide produced bears a single modifying group on the light chain of the peptide. In another embodiment, the method provides a modified peptide that bears a single modifying  
10 group on the heavy chain. In still another embodiment, the method provides a modified peptide with a single modifying group on the light chain and a single modifying group on the heavy chain.

[0399] In another aspect, the invention provides a method of preparing a modified peptide. The method includes contacting the peptide with a modified sugar donor bearing a modifying  
15 group and an enzyme capable of transferring a modified sugar moiety from the modified sugar donor onto an amino acid or glycosyl residue of the peptide.

[0400] In an exemplary embodiment, the method provides a population of modified peptides in which at least 40%, preferably at least 50%, preferably at least 60%, more preferably at least 70% and even more preferably at least 80% of the population members are  
20 mono-conjugated on the light chain of the peptide.

[0401] In an exemplary embodiment, the method provides a population of modified peptides in which at least 40%, preferably at least 50%, preferably at least 60%, more preferably at least 70% and even more preferably at least 80% of the population members are di-conjugated on the light chain of the peptide.

[0402] In an exemplary embodiment of this aspect, the method provides a population of modified peptides in which no more than 50%, preferably no more than 30%, preferably no more than 20%, more preferably no more than 10% of the population members are mono-conjugated on the heavy chain of the peptide.  
25

[0403] In an exemplary embodiment of this aspect, the method provides a population of modified peptides in which no more than 50%, preferably no more than 30%, preferably no  
30

more than 20%, more preferably no more than 10% of the population members are di-conjugated on the heavy chain of the peptide.

[0404] The peptide can be subjected to the action of a sialidase prior to the contacting step, or the peptide can be used without prior desialylation. When the peptide is contacted with a sialidase it can be either essentially completely desialylated or only partially desialylated. In a preferred embodiment, the peptide is at least partially desialylated prior to the contacting step. The peptide may be essentially completely desialylated (essentially asialo) or only partially desialylated. In a preferred embodiment, the desialylated peptide is one of the desialylated embodiments described hereinabove.

### 10 **III. D. Additional aliquots of reagents added in the synthesis of Peptide Conjugates**

[0405] In an exemplary embodiment of the synthesis of the peptide conjugates described herein, one or more additional aliquots of a reaction component/reagent is added to the reaction mixture after a selected period of time. In an exemplary embodiment, the peptide conjugate is a peptide conjugate. In another exemplary embodiment, the reaction component/reagent added is a modified sugar nucleotide. Introduction of a modified sugar nucleotide into the reaction will increase the likelihood of driving the GlycoPEGylation reaction to completion. In an exemplary embodiment, the nucleotide sugar is a CMP-SA-PEG described herein. In an exemplary embodiment, the reaction component/reagent added is a sialidase. In an exemplary embodiment, the reaction component/reagent added is a glycosyltransferase. In an exemplary embodiment, the reaction component/reagent added is magnesium. In an exemplary embodiment, the additional aliquot added represents about 10%, or 20%, or 30%, or 40%, or 50%, or 60%, or 70%, or 80% or 90% of the original amount in added at the start of the reaction. In an exemplary embodiment, the reaction component/reagent is added to the reaction about 3 hours, or 6 hours, or 8 hours, or 10 hours, or 12 hours, or 18 hours, or 24 hours, or 30 hours, or 36 hours after its start.

### **III. E. Purification of Peptide Conjugates**

[0406] The products produced by the above processes can be used without purification. However, it is usually preferred to recover the product and one or more of the intermediates, e.g., nucleotide sugars, branched and linear PEG species, modified sugars and modified nucleotide sugars. Standard, well-known techniques for recovery of glycosylated peptides such as thin or thick layer chromatography, column chromatography, ion exchange chromatography, or membrane filtration can be used. It is preferred to use membrane

filtration, more preferably utilizing a reverse osmotic membrane, or one or more column chromatographic techniques for the recovery as is discussed hereinafter and in the literature cited herein. For instance, membrane filtration wherein the membranes have molecular weight cutoff of about 3000 to about 10,000 can be used to remove proteins such as glycosyl transferases. In certain instances, the molecular weight cutoff differences between the impurity and the product will be utilized in order to ensure product purification. For example, in order to purify product peptide-SA-PEG-40 kD from unreacted CMP-SA-PEG-40 kD, a filter must be chosen that will allow, for example, peptide-SA-PEG-40 kD to remain in the retentate while allowing CMP-SA-PEG-40 kD to flow into the filtrate. Nanofiltration or reverse osmosis can then be used to remove salts and/or purify the product saccharides (*see, e.g.*, WO 98/15581). Nanofilter membranes are a class of reverse osmosis membranes that pass monovalent salts but retain polyvalent salts and uncharged solutes larger than about 100 to about 2,000 Daltons, depending upon the membrane used. Thus, in a typical application, saccharides prepared by the methods of the present invention will be retained in the membrane and contaminating salts will pass through.

**[0407]** If the peptide is produced intracellularly, as a first step, the particulate debris, either host cells or lysed fragments, is removed. Following glycoPEGylation, the PEGylated peptide is purified by art-recognized methods, for example, by centrifugation or ultrafiltration; optionally, the protein may be concentrated with a commercially available protein concentration filter, followed by separating the polypeptide variant from other impurities by one or more steps selected from immunoaffinity chromatography, ion-exchange column fractionation (*e.g.*, on diethylaminoethyl (DEAE) or matrices containing carboxymethyl or sulfopropyl groups), chromatography on Blue-Sepharose, CM Blue-Sepharose, MONO-Q, MONO-S, lentil lectin-Sepharose, WGA-Sepharose, Con A-Sepharose, Ether Toyopearl, Butyl Toyopearl, Phenyl Toyopearl, or protein A Sepharose, SDS-PAGE chromatography, silica chromatography, chromatofocusing, reverse phase HPLC (*e.g.*, silica gel with appended aliphatic groups), gel filtration using, *e.g.*, Sephadex molecular sieve or size-exclusion chromatography, chromatography on columns that selectively bind the polypeptide, and ethanol or ammonium sulfate precipitation. Purification can be used to separate one chain of the Factor VII/Factor VIIa peptide conjugate from the other, as further described later in this section.

**[0408]** Modified glycopeptides produced in culture are usually isolated by initial extraction from cells, enzymes, etc., followed by one or more concentration, salting-out, aqueous ion-

exchange, or size-exclusion chromatography steps. Additionally, the modified glycoprotein may be purified by affinity chromatography. Finally, HPLC may be employed for final purification steps.

[0409] A protease inhibitor may be included in any of the foregoing steps to inhibit proteolysis and antibiotics or preservatives may be included to prevent the growth of adventitious contaminants. The protease inhibitors used in the foregoing steps may be low molecular weight inhibitors, including antipain, alpha-1-antitrypsin, anti-thrombin, leupeptin, amastatin, chymostatin, benzamidin, as well as other serine protease inhibitors (i.e. serpins). Generally, serine protease inhibitors should be used in concentrations ranging from 0.5 – 100  $\mu$ M, although chymostatin in cell culture may be used in concentrations upward of 200  $\mu$ M. Other serine protease inhibitors will include inhibitors specific to the chymotrypsin-like, the subtilisin-like, the alpha/beta hydrolase, or the signal peptidase clans of serine proteases. Besides serine proteases, other types of protease inhibitors may also be used, including cysteine protease inhibitors (1 - 10  $\mu$ M) and aspartic protease inhibitors (1 - 5  $\mu$ M), as well as non-specific protease inhibitors such as pepstatin (.1 – 5  $\mu$ M). Protease inhibitors used in this invention may also include natural protease inhibitors, such as the hirustasin inhibitor isolated from leech. In some embodiments, protease inhibitors will comprise synthetic peptides or antibodies that are able to bind with specificity to the protease catalytic site to stabilize Factor VII/Factor VIIa without interfering with a glycoPEGylation reaction.

[0410] Within another embodiment, supernatants from systems which produce the modified glycopeptide of the invention are first concentrated using a commercially available protein concentration filter, for example, an Amicon or Millipore Pellicon ultrafiltration unit. Following the concentration step, the concentrate may be applied to a suitable purification matrix. For example, a suitable affinity matrix may comprise a ligand for the peptide, a lectin or antibody molecule bound to a suitable support. Alternatively, an anion-exchange resin may be employed, for example, a matrix or substrate having pendant DEAE groups. Suitable matrices include acrylamide, agarose, dextran, cellulose, or other types commonly employed in protein purification. Alternatively, a cation-exchange step may be employed. Suitable cation exchangers include various insoluble matrices comprising sulfopropyl or carboxymethyl groups. Sulfopropyl groups are particularly preferred.

[0411] Other methods of use in purification include size exclusion chromatography (SEC), hydroxyapatite chromatography, hydrophobic interaction chromatography and

chromatography on Blue Sepharose. These and other useful methods are illustrated in co-assigned U.S. Provisional Patent No. (Attorney Docket No. 40853-01-5168-P1, filed May 6, 2005).

[0412] One or more RP-HPLC steps employing hydrophobic RP-HPLC media, *e.g.*, silica gel having pendant methyl or other aliphatic groups, may be employed to further purify a polypeptide conjugate composition. Some or all of the foregoing purification steps, in various combinations, can also be employed to provide a homogeneous or essentially homogeneous modified glycoprotein.

[0413] The modified glycopeptide of the invention resulting from a large-scale fermentation may be purified by methods analogous to those disclosed by Urdal *et al.*, *J. Chromatog.* **296**: 171 (1984). This reference describes two sequential, RP-HPLC steps for purification of recombinant human IL-2 on a preparative HPLC column. Alternatively, techniques such as affinity chromatography may be utilized to purify the modified glycoprotein.

[0414] In an exemplary embodiment, the purification is accomplished by the methods set forth in commonly owned, co-assigned U.S. Provisional Patent No. 60/665,588, filed March 24, 2005.

[0415] According to the present invention, pegylated peptides or peptide conjugate produced either via sequential de-sialylation or simultaneous sialylation can be purified or resolved by using magnesium chloride gradient.

#### **IV. Pharmaceutical Compositions**

[0416] In another aspect, the invention provides a pharmaceutical composition. The pharmaceutical composition includes a pharmaceutically acceptable diluent and a covalent conjugate between a non-naturally-occurring, PEG moiety, therapeutic moiety or biomolecule and a glycosylated or non-glycosylated peptide. The polymer, therapeutic moiety or biomolecule is conjugated to the peptide via an intact glycosyl linking group interposed between and covalently linked to both the peptide and the polymer, therapeutic moiety or biomolecule.

[0417] Pharmaceutical compositions of the invention are suitable for use in a variety of drug delivery systems. Suitable formulations for use in the present invention are found in



*Remington's Pharmaceutical Sciences*, Mace Publishing Company, Philadelphia, PA, 17th ed. (1985). For a brief review of methods for drug delivery, *see*, Langer, *Science* **249**:1527-1533 (1990).

[0418] In an exemplary embodiment, the pharmaceutical formulation comprises a peptide  
5 conjugate and a pharmaceutically acceptable diluent which is a member selected from sodium chloride, calcium chloride dihydrate, glycylglycine, polysorbate 80, and mannitol. In another exemplary embodiment, the pharmaceutically acceptable diluent is sodium chloride and glycylglycine. In another exemplary embodiment, the pharmaceutically acceptable diluent is calcium chloride dihydrate and polysorbate 80. In another exemplary embodiment, the  
10 pharmaceutically acceptable diluent is mannitol.

[0419] The pharmaceutical compositions may be formulated for any appropriate manner of administration, including for example, topical, oral, nasal, intravenous, intracranial, intraperitoneal, subcutaneous or intramuscular administration. For parenteral administration, such as subcutaneous injection, the carrier preferably comprises water, saline, alcohol, a fat, a  
15 wax or a buffer. For oral administration, any of the above carriers or a solid carrier, such as mannitol, lactose, starch, magnesium stearate, sodium saccharine, talcum, cellulose, glucose, sucrose, and magnesium carbonate, may be employed. Biodegradable microspheres (*e.g.*, polylactate polyglycolate) may also be employed as carriers for the pharmaceutical compositions of this invention. Suitable biodegradable microspheres are disclosed, for  
20 example, in U.S. Patent Nos. 4,897,268 and 5,075,109.

[0420] Commonly, the pharmaceutical compositions are administered parenterally, *e.g.*, intravenously. Thus, the invention provides compositions for parenteral administration that include the compound dissolved or suspended in an acceptable carrier, preferably an aqueous carrier, *e.g.*, water, buffered water, saline, PBS and the like. The compositions may contain  
25 pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions, such as pH adjusting and buffering agents, tonicity adjusting agents, wetting agents, detergents and the like.

[0421] These compositions may be sterilized by conventional sterilization techniques, or may be sterile filtered. The resulting aqueous solutions may be packaged for use as is, or  
30 lyophilized, the lyophilized preparation being combined with a sterile aqueous carrier prior to administration. The pH of the preparations typically will be between 3 and 11, more preferably from 5 to 9 and most preferably from 7 and 8.

[0422] In some embodiments the glycopeptides of the invention can be incorporated into liposomes formed from standard vesicle-forming lipids. A variety of methods are available for preparing liposomes, as described in, *e.g.*, Szoka *et al.*, *Ann. Rev. Biophys. Bioeng.* **9**: 467 (1980), U.S. Pat. Nos. 4,235,871, 4,501,728 and 4,837,028. The targeting of liposomes using  
5 a variety of targeting agents (*e.g.*, the sialyl galactosides of the invention) is well known in the art (*see, e.g.*, U.S. Patent Nos. 4,957,773 and 4,603,044).

[0423] Standard methods for coupling targeting agents to liposomes can be used. These methods generally involve incorporation into liposomes of lipid components, such as phosphatidylethanolamine, which can be activated for attachment of targeting agents, or  
10 derivatized lipophilic compounds, such as lipid-derivatized glycopeptides of the invention.

[0424] Targeting mechanisms generally require that the targeting agents be positioned on the surface of the liposome in such a manner that the target moieties are available for interaction with the target, for example, a cell surface receptor. The carbohydrates of the invention may be attached to a lipid molecule before the liposome is formed using methods  
15 known to those of skill in the art (*e.g.*, alkylation or acylation of a hydroxyl group present on the carbohydrate with a long chain alkyl halide or with a fatty acid, respectively).

Alternatively, the liposome may be fashioned in such a way that a connector portion is first incorporated into the membrane at the time of forming the membrane. The connector portion must have a lipophilic portion, which is firmly embedded and anchored in the membrane. It  
20 must also have a reactive portion, which is chemically available on the aqueous surface of the liposome. The reactive portion is selected so that it will be chemically suitable to form a stable chemical bond with the targeting agent or carbohydrate, which is added later. In some cases it is possible to attach the target agent to the connector molecule directly, but in most instances it is more suitable to use a third molecule to act as a chemical bridge, thus linking  
25 the connector molecule which is in the membrane with the target agent or carbohydrate which is extended, three dimensionally, off of the vesicle surface.

[0425] The compounds prepared by the methods of the invention may also find use as diagnostic reagents. For example, labeled compounds can be used to locate areas of inflammation or tumor metastasis in a patient suspected of having an inflammation. For this  
30 use, the compounds can be labeled with  $^{125}\text{I}$ ,  $^{14}\text{C}$ , or tritium.

[0426] Preparative methods for species of use in preparing the compositions of the invention are generally set forth in various patent publications, *e.g.*, US 20040137557; WO

04/083258; and WO 04/033651. The following examples are provided to illustrate the conjugates, and methods and of the present invention, but not to limit the claimed invention.

## EXAMPLES

### EXAMPLE 1

#### 5 *Desialylation of Factor VIIa.*

[0427] Factor VIIa which was expressed in serum-free media, Factor VIIa which was produced in serum containing media, plus three Factor VIIa mutants N145Q, N322Q, and analogue DVQ (V158D/E296V/M298Q).

[0428] In preparation for enzymatic desialylation, Factor VIIa was dialyzed into MES,  
10 150 mM NaCl, 5 mM CaCl<sub>2</sub>, 50mM MES, pH 6 overnight at 4°C in Snakeskin dialysis tubing with a MWCO of 10 kD. Desialylation of Factor VIIa (1 mg/mL) was performed with 10 U/L soluble sialidase from *Arthrobacter ureafaciens* (Calbiochem) at 32°C for 18 hours in the exchanged buffer.

### EXAMPLE 2

#### 15 *Sialyl-PEGylation of Factor VIIa.*

[0429] Sialyl-PEGylation ("GlycoPEGylation") was performed on asialo-Factor VIIa (1 mg/mL) with 100 U/L ST3Gal-III and 200 μM CMP-sialic acid-PEG (40 kD, 20 kD, 10 kD, 5 kD, and 2 kD) at 32°C in the desialylation buffer for 2-6 hours. After the proper reaction time had expired, the PEGylated sample was immediately purified to minimize further  
20 GlycoPEGylation.

[0430] To cap GlycoPEGylated Factor VII/Factor VIIa with samples capped with sialic acid, the sialidase was first removed from the asialo-Factor VIIa by anion-exchange chromatography as indicated below. Excess CMP-sialic acid (5 mM) was added and incubated at 32°C for 2 hours, capping GlycoPEGylated Factor VIIa with sialic acid.

25 The sialyl-PEGylated forms of Factor VIIa were analyzed by non-reducing SDS-PAGE (Tris-glycine gels and/or NuPAGE gels) and a Colloidal Blue Staining Kit, as described by Invitrogen.

**EXAMPLE 3***Purification of PEGylated Factor VIIa.*

[0431] GlycoPEGylated samples of Factor VIIa were purified with a modified anion-exchange method. Samples were handled at 5°C. Immediately before loading the column, 1 g Chelex 100 (BioRad) per 10 mL Factor VIIa solution was added to the remodeled sample. After stirring for 10 min, the suspension was filtered on a cellulose acetate membrane (0.2 µm) with a vacuum system. The retained chelator resin on the filter was washed once with 1-2 mL water per 10 mL bulk. The conductivity of the filtrate was adjusted to 10 mS/cm at 5°C, and adjusted to pH 8.6, if necessary.

[0432] Anion exchange was performed at 8-10°C. A column containing Q Sepharose FF was prepared before loading by washing with 1 M NaOH (10 column volumes), water (5 column volumes), 2 M NaCl, 50 mM HOAc, pH 3 (10 column volumes), and equilibrating with 175 mM NaCl, 10 mM glycylglycine, pH 8.6 (10 column volumes). For each PEGylation reaction, 15-20 mg Factor VIIa was loaded on to an XK16 column (Amersham Biosciences) with 10 mL Q Sepharose FF (no more than 2 mg protein per mL resin) at a flow rate of 100 cm/h. For the 2 kD linear PEG, 20 mg Factor VIIa was loaded on to an XK26 column (Amersham Biosciences) with 40 mL Q Sepharose FF (0.5 mg protein per mg resin) at a flow rate of 100 cm/h.

[0433] After loading, the column was washed with 175 mM NaCl, 10 mM glycylglycine, pH 8.6 (10 column volumes) and 50 mM NaCl, 10 mM glycylglycine, pH 8.6 (2 column volumes). Elution was performed with a step gradient of 15 mM CaCl<sub>2</sub> by using 50 mM NaCl, 10 mM glycylglycine, 15 mM CaCl<sub>2</sub>, pH 8.6 (5 column volumes). The column was then washed with 1 M NaCl, 10 mM glycylglycine, pH 8.6 (5 column volumes). The effluent was monitored by absorbance at 280 nm. Fractions (5 mL) were collected during the flow-through and the two washes; 2.5 mL fractions were collected during the CaCl<sub>2</sub> and 1M salt elutions. Fractions containing Factor VIIa were analyzed by non-reducing SDS-PAGE (Tris-glycine gels and/or NUPAGE gels) and a Colloidal Blue Staining Kit. The appropriate fractions with Factor VIIa were pooled, and the pH was adjusted to 7.2 with 4 M HCl.

[0434] Factor VIIa-SA-PEG-10 kD was purified as described above, except for the following changes. EDTA (10 mM) was added to the PEGylated Factor VIIa solution, the pH was adjusted to pH 6, and the conductivity was adjusted to 5mS/cm, at 5°C. About

20 mg of Factor VIIa-SA-PEG-10 kD was loaded on to an XK16 column (Amersham Biosciences) with 10 mL Poros 50 Micron HQ resin (no more than 2 mg protein per mL, resin) at a flow rate of 100 cm/h. After loading, the column was washed with 175 mM NaCl, 10 mM histidine pH 6 (10 column volumes) and 50 mM NaCl, 10 mM histidine, pH 6 (2 column volumes). Elution was performed with a step gradient of 20 mM CaCl<sub>2</sub> in 50 mM NaCl, 10 mM histidine, pH 6 (5 column volumes). The column was then washed with 1 M NaCl, 10 mM histidine, pH 6 (5 column volumes).

[0435] The anion-exchange eluate containing Factor VIIa-SA-PEG-10 kD (25mL) was concentrated to 5-7 mL by using an Amicon Ultra-15 10K centrifugal filter device, according to the manufacturer's directions (Millipore). Following concentration, size exclusion chromatography was performed. The sample (5-7 mL) was loaded onto a column containing Superdex 200 (HiLoad 16/60, prep grade; Amersham Biosciences) equilibrated in 50 mM NaCl, 10 mM glycylglycine, 15 mM CaCl<sub>2</sub>, pH 7.2 for most of the PEGylated variants. Factor VIIa-SA-PEG-10 kD was separated from the unmodified, asialo-Factor VIIa at a flow rate of 1 mL/min, and the absorbance was monitored at 280 nm. Fractions (1 mL) containing Factor VIIa were collected and analyzed by non-reducing SDS-PAGE (Tris-glycine gels and/or NuPAGE gels) and a Colloidal Blue Staining Kit. Fractions containing the targeted PEGylated isoform and devoid of the unmodified, asialo-Factor VIIa were pooled and concentrated to 1 mg/mL using an Amicon Ultra-15 10K centrifugal filter device. Protein concentration was determined from absorbance readings at 280 nm using an extinction coefficient of 1.37 (mg/mL)<sup>-1</sup>cm<sup>-1</sup>.

#### EXAMPLE 4

*Determination of PEGylated Isoforms by Reversed phase HPLC analysis.*

[0436] PEGylated Factor VIIa was analyzed by HPLC on a reversed-phase column (Zorbax 300SB-C3, 5 µm particle size, 2.1 x 150 mm). The eluants were A) 0.1 TFA in water and B) 0.09 % TFA in acetonitrile. Detection was at 214 nm. The gradient, flow rate, and column temperature depended on the PEG length (40 kD, 20 kD, and 10 kD PEG: 35-65 %B in 30 min, 0.5 mL/min, 45°C; 10 kD PEG: 35-60 %B in 30 min, 0.5 mL/min, 45°C; 5 kD: 40-50 %B in 40 min, 0.5 mL/min, 45°C; 2 kD: 38-43 %B in 67 min, 0.6 mL/min, 55°C). The identity of each peak was assigned based on two or more of four different pieces of evidence: the known retention time of native Factor VIIa, the SDS-PAGE migration of the isolated peak, the MALDI-TOF mass

spectrum of the isolated peak, and the orderly progression of the retention time of each peak with increasing number of attached PEG.

### EXAMPLE 5

#### *Determination of Site of PEG Attachment by Reversed-phase HPLC.*

- 5    **[0437]**     Factor VIIa and PEGylated Factor VIIa variants were reduced by mixing sample (10  $\mu$ L at a concentration of 1 mg/mL) with reducing buffer (40  $\mu$ L, 50 mM NaCl, 10 mM glycylglycine, 15 mM EDTA, 8 M urea, 20 mM DTT, pH 8.6) for 15 min at room temperature. Water (50  $\mu$ L) was added and the sample cooled to 4°C until injected on the HPLC (< 12 hrs). The HPLC column, eluants, and detection were as described above for  
10   non-reduced samples. The flow rate was 0.5 mL/min and the gradient was 30-55 %B in 90 min, followed by a brief wash cycle up to 90 %B. The identity of each peak was assigned as described in Example 4.

### EXAMPLE 6

#### *Factor VIIa Clotting Assay.*

- 15   **[0438]**     PEGylated samples and standards were tested in duplicate, and were diluted in 100mM NaCl, 5mM CaCl<sub>2</sub>. 0.1% BSA (wt/vol), 50mM Tris, pH 7.4. The standard and samples were assayed over a range from 0.1 to 10 ng/mL. Equal volumes of diluted standards and samples were mixed with Factor VIIa deficient plasma (Diagnostica Stago), and stored on ice for no greater than 4 hours before they were assayed.
- 20   **[0439]**     Clotting times were measured with a STart4 coagulometer (Diagnostica Stago). The coagulometer measured the time elapsed until an *in vitro* clot was formed, as indicated by the stopping of the gentle back-and-forth movement of a magnetic ball in a sample cuvette.
- 25   **[0440]**     Into each cuvette, one magnetic ball was deposited, plus 100  $\mu$ L Factor VIIa sample/deficient plasma and 100  $\mu$ L of a diluted rat brain cephalin solution (stored on ice for no greater than 4 hours). Each reagent was added with 5 seconds between each well, and the final mixture was incubated for 300 seconds at 37°C. Diluted rat brain cephalin (RBC) solution was made from 2 mL RBC stock solution (1 vial RBC stock, from Haemachem, plus 10 mL 150mM NaCl) and 4 mL 100mM NaCl, 5mM CaCl<sub>2</sub>, 0.1% BSA (wt/vol), 50mM  
30   Tris, pH 7.4.

[0441] At 300 seconds, the assay was started by the addition of 100  $\mu$ L of a pre-heated (37°C) solution of soluble tissue factor (2 $\mu$ g/mL; amino acids 1-209) in 100mM NaCl. 12.5mM CaCl<sub>2</sub>, 0.1% BSA (wt/vol), 50mM Tris, pH 7.4. Again, this next solution was added with a 5 second interval between samples.

- 5 [0442] The clotting times from the diluted standards were used to generate a standard curve (log clot time versus log Factor VIIa concentration). The resulting linear regression from the curve was used to determine the relative clotting activities of PEGylated variants. PEGylated Factor VIIa variants were compared against an aliquotted stock of Factor VIIa.

### EXAMPLE 7

10 *GlycoPEGylation of Recombinant Factor VIIa produced in BHK cells*

[0443] This example sets forth the PEGylation of recombinant Factor VIIa made in BHK cells.

- [0444] *Preparation of Asialo-Factor VIIa.* Recombinant Factor VIIa was produced in BHK cells (baby hamster kidney cells). Factor VIIa (14.2 mg) was dissolved at 1 mg/mL in  
15 buffer solution (pH 7.4, 0.05 M Tris, 0.15 M NaCl, 0.001 M CaCl<sub>2</sub>, 0.05% NaN<sub>3</sub>) and was incubated with 300 mU/mL sialidase (*Vibrio cholera*)-agarose conjugate for 3 days at 32 °C. To monitor the reaction a small aliquot of the reaction was diluted with the appropriate buffer and an IEF gel performed according to Invitrogen procedures (Figure 157). The mixture was centrifuged at 3,500 rpm and the supernatant was collected. The resin was washed three  
20 times (3 $\times$ 2 mL) with the above buffer solution (pH 7.4, 0.05 M Tris, 0.15 M NaCl, 0.05% NaN<sub>3</sub>) and the combined washes were concentrated in a Centricon-Plus-20. The remaining solution was buffer exchanged with 0.05 M Tris (pH 7.4), 0.15 M NaCl, 0.05% NaN<sub>3</sub> to a final volume of 14.4 mL.

- [0445] *Preparation of Factor VIIa-SA-PEG-1kD and Factor VIIa-SA-PEG-10 kD.* The  
25 desialylation of Factor VIIa solution was split into two equal 7.2 mL samples. To each sample was added either CMP-SA-PEG-1 kD (7.4 mg) or CMP-SA-PEG-10 kD (7.4 mg). ST3Gal3 (1.58U) was added to both tubes and the reaction mixtures were incubated at 32°C for 96 hrs. The reaction was monitored by SDS-PAGE gel using reagents and conditions described by Invitrogen. When the reaction was complete, the reaction mixture was purified  
30 using a Toso Haas TSK-Gel-3000 preparative column using PBS buffer (pH 7.1) and collecting fractions based on UV absorption. The combined fractions containing the product

were concentrated at 4°C in Centricon-Plus-20 centrifugal filters (Millipore, Bedford, MA) and the concentrated solution reformulated to yield 1.97 mg (bicinchoninic acid protein assay, BCA assay, Sigma-Aldrich, St. Louis MO) of Factor VIIa-SA-PEG. The product of the reaction was analyzed using SDS-PAGE and IEF analysis according to the procedures and reagents supplied by Invitrogen. Samples were dialyzed against water and analyzed by MALDI-TOF.

### EXAMPLE 8

#### *Factor VIIa-SA-PEG-10kD: One Pot Method*

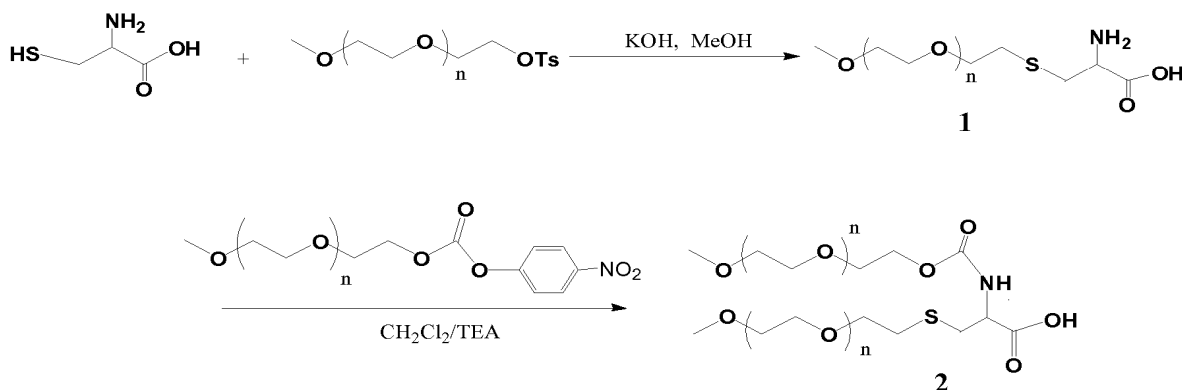
[0446] Factor VIIa (5 mg diluted in the product formulation buffer to a final concentration of 1 mg/mL), CMP-SA-PEG-10 kD (10mM, 60 µL) and *A. niger* enzyme ST3Gal3 (33 U/L) and 10 mM histidine, 50 mM NaCl, 20 mM CaCl<sub>2</sub> were combined in a reaction vessel along with either 10 U/L, 1 U/L, 0.5 U/L or 0.1 U/L of sialidase (CalBiochem). The ingredients were mixed and incubated at 32°C. Reaction progress was measured by analyzing aliquots at 30 minute intervals for the first four hours. An aliquot was then removed at the 20 hour timepoint and subjected to SDS-PAGE. Extent of PEGylation was determined by removing 1 mL at 1.5, 2.5 and 3.5 hour timepoint and purifying the sample on a Poros 50HQ column.

[0447] For the reaction conditions containing 10 U/L of sialidase, no appreciable amount of Factor VIIa-SA-PEG product was formed. For the reaction conditions containing 1 U/L of sialidase, about 17.6 % of the Factor VIIa in the reaction mixture was either mono or diPEGylated after 1.5 hours. This increased to 29% after 2.5 hours, and 40.3% after 3.5 hours. For the reaction conditions containing 0.5 U/L of sialidase, about 44.5 % of the Factor VIIa in the reaction mixture was either mono or diPEGylated after 3 hours, and 0.8% was triPEGylated or greater. After 20 hours, 69.4% was either mono or diPEGylated, and 18.3% was triPEGylated or greater.

[0448] For the reaction conditions containing 0.1 U/L of sialidase, about 29.6% of the Factor VIIa in the reaction mixture was either mono or diPEGylated after 3 hours. After 20 hours, 71.3% was either mono or diPEGylated, and 15.1% was triPEGylated or greater.



## EXAMPLE 9

*Preparation of Cysteine-PEG<sub>2</sub> ( 2 )**a. Synthesis of Compound 1*

5 [0449] Potassium hydroxide (84.2 mg, 1.5 mmol, as a powder) was added to a solution of L-cysteine (93.7mg, 0.75 mmol) in anhydrous methanol (20 L) under argon. The mixture was stirred at room temperature for 30 min, and then mPEG-O-tosylate of molecular mass 20 kilodalton (Ts; 1.0 g, 0.05 mmol) was added in several portions over 2 hours. The mixture was stirred at room temperature for 5 days, and concentrated by rotary evaporation. The residue was diluted with water (30 mL), and stirred at room temperature for 2 hours to destroy any excess 20 kilodalton mPEG-O-tosylate. The solution was then neutralized with acetic acid, the pH adjusted to pH 5.0 and loaded onto a reverse phase chromatography (C-18 silica) column. The column was eluted with a gradient of methanol/water (the product elutes at about 70% methanol), product elution monitored by evaporative light scattering, and the appropriate fractions collected and diluted with water (500 mL). This solution was chromatographed (ion exchange, XK 50 Q, BIG Beads, 300 mL, hydroxide form; gradient of water to water/acetic acid-0.75N) and the pH of the appropriate fractions lowered to 6.0 with acetic acid. This solution was then captured on a reversed phase column (C-18 silica) and eluted with a gradient of methanol/water as described above. The product fractions were pooled, concentrated, redissolved in water and freeze-dried to afford 453 mg (44%) of a white solid (1).

15 [0450] Structural data for the compound were as follows: <sup>1</sup>H-NMR (500 MHz; D<sub>2</sub>O) δ 2.83 (t, 2H, O-C-CH<sub>2</sub>-S), 3.05 (q, 1H, S-CH<sub>2</sub>-CHN), 3.18 (q, 1H, (q, 1H, S-CH<sub>2</sub>-CHN), 3.38 (s, 3H, CH<sub>3</sub>O), 3.7 (t, OCH<sub>2</sub>CH<sub>2</sub>O), 3.95 (q, 1H, CHN). The purity of the product was confirmed by SDS PAGE.

*b. Synthesis of Cysteine-PEG<sub>2</sub> (2)*

[0451] Triethylamine (~0.5 mL) was added dropwise to a solution of compound 1 (440 mg, 22  $\mu$ mol) dissolved in anhydrous CH<sub>2</sub>Cl<sub>2</sub> (30 mL) until the solution was basic. A solution of 20 kilodalton mPEG-O-p-nitrophenyl carbonate (660 mg, 33  $\mu$ mol) and N-hydroxysuccinimide (3.6 mg, 30.8  $\mu$ mol) in CH<sub>2</sub>Cl<sub>2</sub> (20 mL) was added in several portions over 1 hour at room temperature. The reaction mixture was stirred at room temperature for 24 hours. The solvent was then removed by rotary evaporation, the residue was dissolved in water (100 mL), and the pH adjusted to 9.5 with 1.0 N NaOH. The basic solution was stirred at room temperature for 2 hours and was then neutralized with acetic acid to a pH 7.0. The solution was then loaded onto a reversed phase chromatography (C-18 silica) column. The column was eluted with a gradient of methanol/water (the product elutes at about 70% methanol), product elution monitored by evaporative light scattering, and the appropriate fractions collected and diluted with water (500 mL). This solution was chromatographed (ion exchange, XK 50 Q, BIG Beads, 300 mL, hydroxide form; gradient of water to water/acetic acid-0.75N) and the pH of the appropriate fractions lowered to 6.0 with acetic acid. This solution was then captured on a reversed phase column (C-18 silica) and eluted with a gradient of methanol/water as described above. The product fractions were pooled, concentrated, redissolved in water and freeze-dried to afford 575 mg (70 %) of a white solid (2).

[0452] Structural data for the compound were as follows: <sup>1</sup>H-NMR (500 MHz; D<sub>2</sub>O)  $\delta$  2.83 (t, 2H, O-C-CH<sub>2</sub>-S), 2.95 (t, 2H, O-C-CH<sub>2</sub>-S), 3.12 (q, 1H, S-CHH-CHN), 3.39 (s, 3H CH<sub>3</sub>O), 3.71 (t, OCH<sub>2</sub>CH<sub>2</sub>O). The purity of the product was confirmed by SDS PAGE.

**EXAMPLE 10***Factor VIIa-SA-PEG-40kD*

[0453] *GlycoPEGylation of Factor VIIa (One Pot with Capping).* GlycoPEGylation of Factor VIIa was accomplished in a one-pot reaction where desialation and PEGylation occur simultaneously, followed by capping with sialic acid. The reaction was performed in a jacketed glass vessel controlled at 32°C by a recirculating waterbath. First, the concentrated 0.2 $\mu$ m-filtered Factor VIIa was introduced into the vessel and heated to 32°C by mixing with a stir bar for 20 minutes. A solution of sialidase was made from dry powder in 10mM histidine/50mM NaCl/20mM CaCl<sub>2</sub>, pH 6.0 at a concentration of 4,000 U/L. Once the Factor VIIa reached 32°C, the sialidase was added to the Factor VIIa, and the reaction was mixed for

approximately 5 minutes to ensure a uniform solution after time which the mixing was stopped. The desialation was allowed to proceed for 1.0 h at 32°C. During the desialation reaction, the CMP-SA-PEG-40 kD was dissolved into 10mM histidine/50mM NaCl/20mM CaCl<sub>2</sub>, pH 6.0 buffer, and the concentration of was determined by UV absorbance at 271nm.

5 After the CMP-SA-PEG-40 kD was dissolved, the CMP-SA-PEG-40 kD was added to the reaction, as well as the ST3Gal3, and the reaction was mixed for approximately 15 minutes with a stir bar to ensure a uniform solution. An additional volume of 85mL of buffer was added to make the reaction 1.0 L. The reaction was allowed to proceed without stirring for 24 hours before CMP-SA was added to a concentration of 4.3 mM to quench the reaction and  
10 cap the remaining terminal galactose residues with sialic acid. The quenching was allowed to proceed with mixing for 30 minutes at 32°C. The total volume of the reaction was 1.0 L before quenching. Timepoint samples (1 mL) were taken at 0, 4.5, 7.5, and 24 h, quenched with CMP-SA, and analyzed by RP-HPLC and SDS-PAGE.

[0454] *Purification of Factor VIIa-SA-PEG-40kD.* After capping, the solution was  
15 diluted with 2.0 L of 10mM histidine, pH 6.0 that had been stored overnight at 4 °C and the sample was filtered through a 0.2µm Millipak 60 filter. The resulting load volume was 3.1 L. The AEX2 chromatography was performed at 20-25°C (ambient room temperature) on an Akta Pilot system. After loading, a 10 column volumes wash with equilibration buffer was performed, and the product was eluted from the column using a 10 column volume gradient  
20 of MgCl<sub>2</sub> which resulted in resolution of PEGylated-Factor VIIa species from unPEGylated Factor VIIa. The loading for this column was intentionally kept low, targeting < 2 mg Factor VIIa/mL resin. SDS-PAGE gels were run in addition to RP-HPLC analysis of selected fractions and pools of fractions in order to make the pool of bulk product. Pooled fractions were pH adjusted to 6.0 with 1M NaOH and stored in the cold room at 2-8°C overnight.

25 [0455] *Final Concentration/Diafiltration, aseptic filtration and aliquoting.* The pooled fractions were filtered through a Millipak 20 0.2µm filter and stored overnight at 2-8°C. To perform the concentration/diafiltration, a Millipore 0.1m<sup>2</sup> 30 kD regenerated cellulose membrane was used in a system fitted with a peristaltic pump and silicone tubing. The system was assembled and flushed with water, then sanitized with 0.1M NaOH for at least 1  
30 hour, and then stored in 0.1M NaOH until equilibration with 10 mM histidine/ 5 mM CaCl<sub>2</sub>/ 100 mM NaCl pH 6.0 diafiltration buffer immediately before use. The product was concentrated to approximately 400 mL and then diafiltered at constant volume with approximately 5 diavolumes of buffer. The product was then concentrated to approximately

300mL and recovered after a low pressure recirculation for 5 minutes, and the membranes were rinsed with 200 mL of diafiltration buffer by a recirculation for 5 minutes. The wash was recovered with product, and another 50mL of buffer was recirculated for another 5 minutes for a final wash. The resulting bulk was approximately 510 mL, and that was filtered through a 1L vacuum filter fitted with a 0.2µm PES membrane (Millipore). The aseptically-filtered bulk was then aliquoted into 25mL aliquots in 50mL sterile falcon tubes and frozen at -80°C.

*Analysis of the PEGylation reaction by HPLC (Example 10)*

	Conjugation Reaction Time				Purification
	0 hrs	4.5 hrs	7.5 hrs	24 hrs	After Chromatography
% Unpegylated	94.7	76.1	66.6	51.0	0.6
% Monopegylated	0.9	17.9	26.1	39.1	85.6
% Dipegylated	0.1	0.9	1.9	5.1	5.1
% Tripegylated	0.0	0.0	0.0	0.2	0.2

After 24 hours, the bulk product PEG-state distribution was: 0.7% unpegylated, 85.3% mono-pegylated, 11.5% di-pegylated, and 0.3% tri-pegylated. Column chromatography is the main step in the process that generates the product distribution, largely through removing unpegylated material from mono- and di-pegylated species.

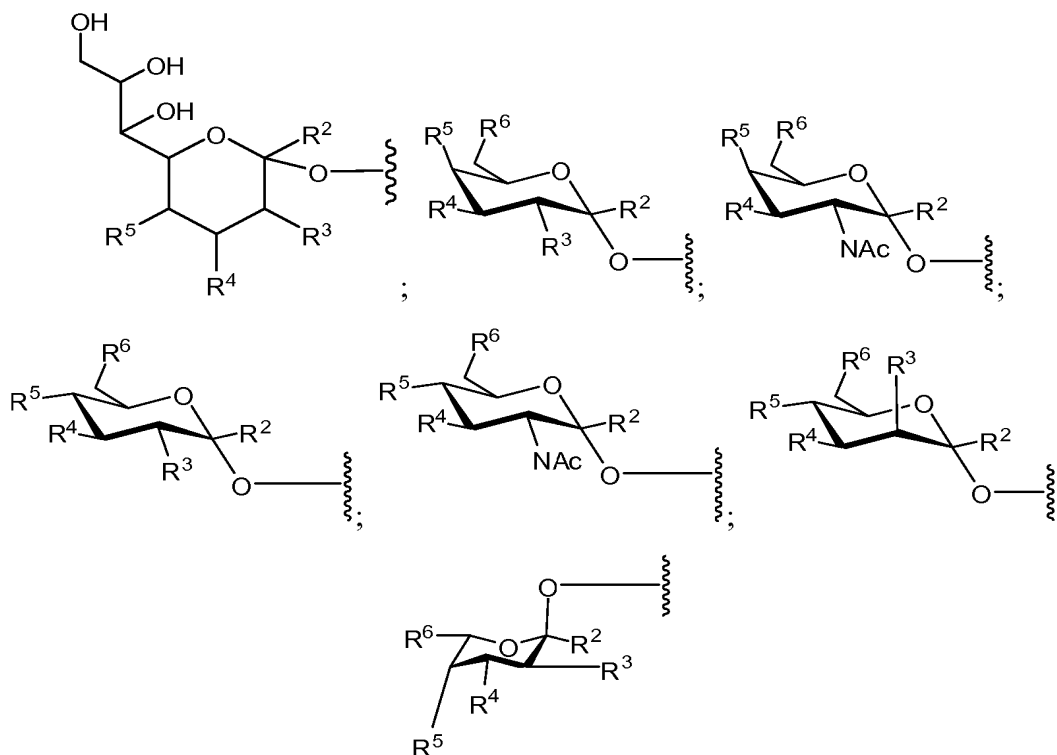
**[0456]** It is understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application and scope of the appended claims. All publications, patents, and patent applications cited herein are hereby incorporated by reference in their entirety for all purposes.

**WHAT IS CLAIMED IS:**

1. A peptide conjugate comprising:

a) a peptide which is covalently attached to a moiety which is a member

selected from:



in which R<sup>2</sup> is a member selected from H, CH<sub>2</sub>OR<sup>7</sup>, COOR<sup>7</sup> and OR<sup>7</sup>,

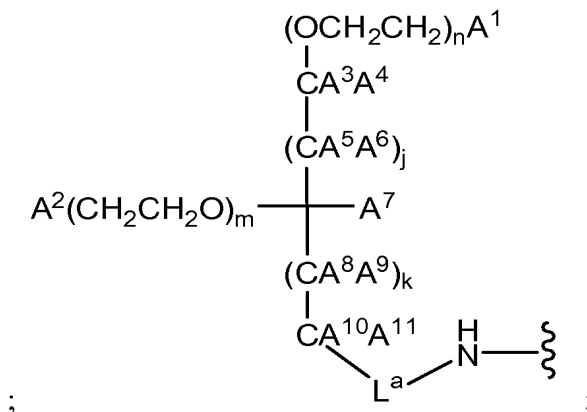
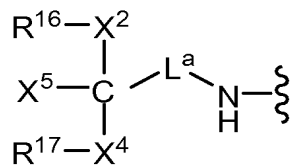
wherein R<sup>7</sup> is a member selected from H, substituted or unsubstituted alkyl and substituted or unsubstituted heteroalkyl;

R<sup>3</sup>, R<sup>4</sup>, R<sup>5</sup> and R<sup>6</sup> are members independently selected from H, substituted or unsubstituted alkyl, OR<sup>8</sup> and NHC(O)R<sup>9</sup>;

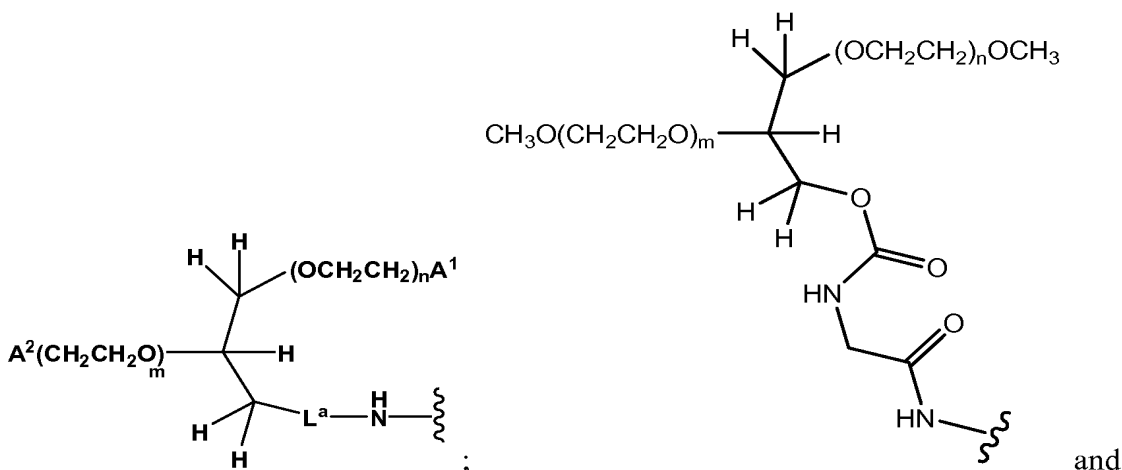
wherein R<sup>8</sup> and R<sup>9</sup> are independently selected from H, substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl, sialic acid and polysialic acid;

and wherein at least one of R<sup>3</sup>, R<sup>4</sup>, R<sup>5</sup>, R<sup>6</sup> includes a moiety which is a member selected from:

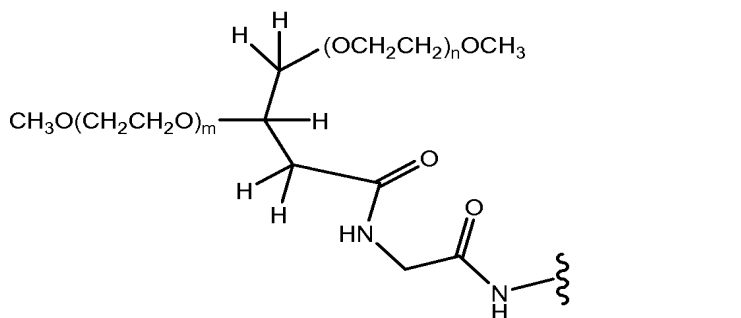
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18



19



20 in which the indices m and n are integers independently selected from 1 to 1000;

21  $A^1, A^2, A^3, A^4, A^5, A^6, A^7, A^8, A^9, A^{10}$  and  $A^{11}$  are members independently selected from

22 H, substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl,

23 substituted or unsubstituted cycloalkyl, substituted or unsubstituted

24 heterocycloalkyl, substituted or unsubstituted aryl, substituted or unsubstituted

25 heteroaryl,  $-NA^{12}A^{13}$ ,  $-OA^{12}$  and  $-SiA^{12}A^{13}$ ;

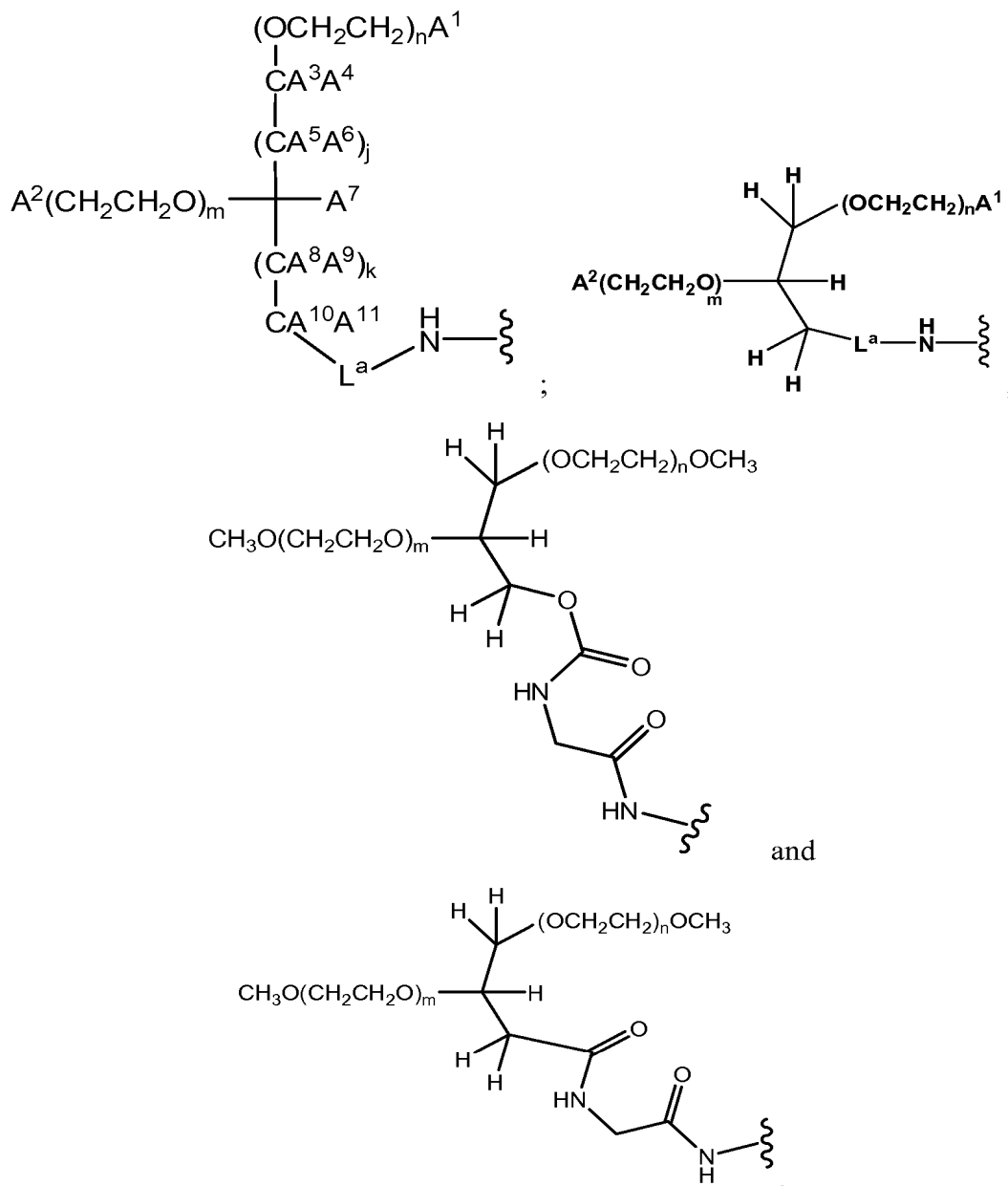
26 wherein

27  $A^{12}$  and  $A^{13}$  are members independently selected from substituted or unsubstituted

28 alkyl, substituted or unsubstituted heteroalkyl, substituted or unsubstituted

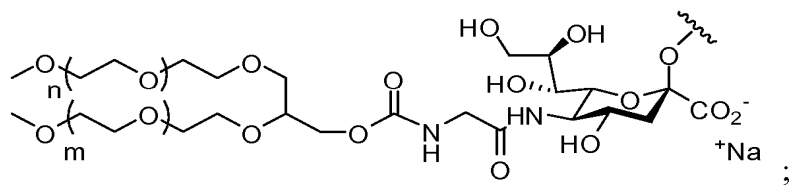
29 cycloalkyl, substituted or unsubstituted heterocycloalkyl, substituted or  
 30 unsubstituted aryl, and substituted or unsubstituted heteroaryl.

1 2. The peptide conjugate of claim 1, wherein said at least one of  $R^3$ ,  
 2  $R^4$ ,  $R^5$ ,  $R^6$  includes a moiety which is a member selected from:

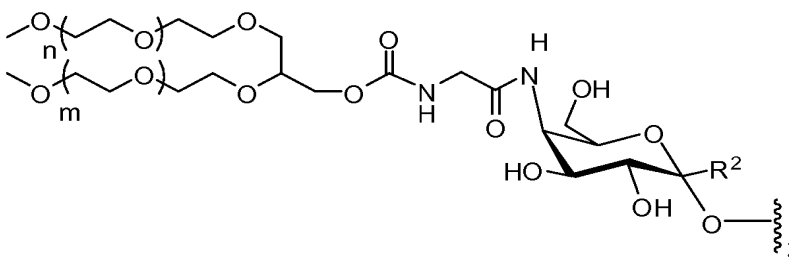


1 3. The peptide conjugate of claim 1, wherein said moiety is a member  
 2 selected from:

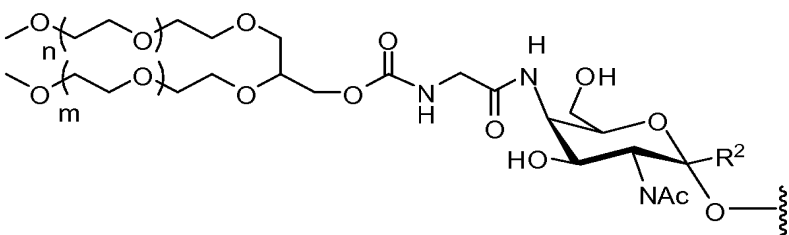
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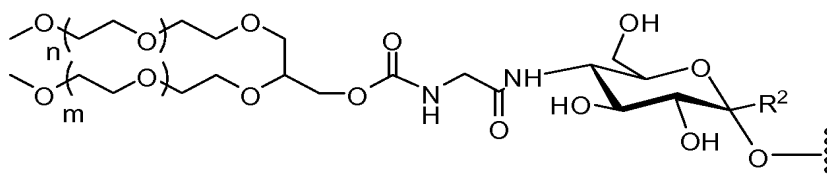
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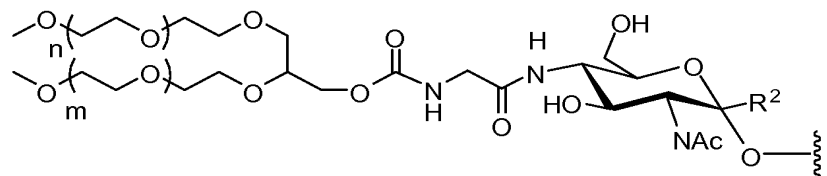
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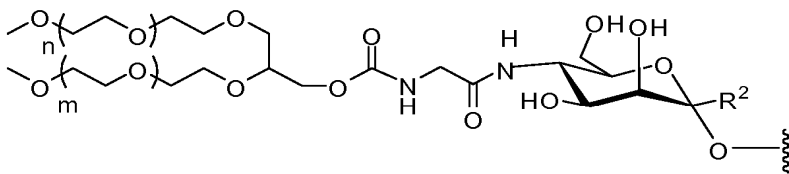
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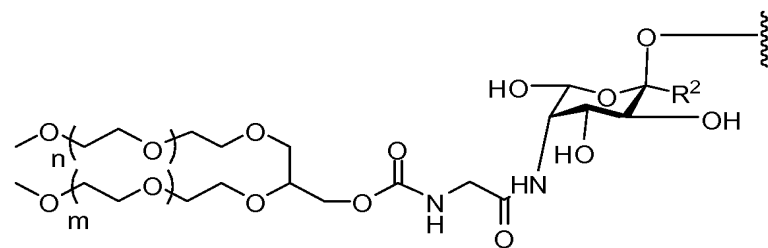
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8



9





1                   4.       The peptide conjugate of claim 1, wherein said peptide in the  
2 peptide conjugate is a member selected from bone morphogenetic protein 2 (BMP-2),  
3 bone morphogenetic protein 7 (BMP-7), bone morphogenetic protein 15 (BMP-15),  
4 neurotrophin-3 (NT-3), von Willebrand factor (vWF) protease, Factor VII, Factor VIIa,  
5 Factor VIII, Factor IX, Factor X, Factor XI, B-domain deleted Factor VIII, vWF-Factor  
6 VIII fusion protein having full-length Factor VIII, vWF-Factor VIII fusion protein having  
7 B-domain deleted Factor VIII, erythropoietin (EPO), granulocyte colony stimulating  
8 factor (G-CSF), Granulocyte-Macrophage Colony Stimulating Factor (GM-CSF),  
9 interferon alpha, interferon beta, interferon gamma,  $\alpha_1$ -antitrypsin (ATT, or  $\alpha_1$ -protease  
10 inhibitor), glucocerebrosidase, Tissue-Type Plasminogen Activator (TPA), Interleukin-2  
11 (IL-2), urokinase, human DNase, insulin, Hepatitis B surface protein (HbsAg), human  
12 growth hormone, TNF Receptor-IgG Fc region fusion protein (Enbrel™), anti-HER2  
13 monoclonal antibody (Herceptin™), monoclonal antibody to Protein F of Respiratory  
14 Syncytial Virus (Synagis™), monoclonal antibody to TNF- $\alpha$  (Remicade™), monoclonal  
15 antibody to glycoprotein IIb/IIIa (Reopro™), monoclonal antibody to CD20 (Rituxan™),  
16 anti-thrombin III (AT III), human Chorionic Gonadotropin (hCG), alpha-galactosidase  
17 (Fabrazyme™), alpha-iduronidase (Aldurazyme™), follicle stimulating hormone, beta-  
18 glucosidase, anti-TNF-alpha monoclonal antibody, glucagon-like peptide-1 (GLP-1),  
19 glucagon-like peptide-2 (GLP-2), beta-glucosidase, alpha-galactosidase A and fibroblast  
20 growth factor

FIGURE 1

# Preparation of CMP-SA-Glycerol-PEG-40kDa

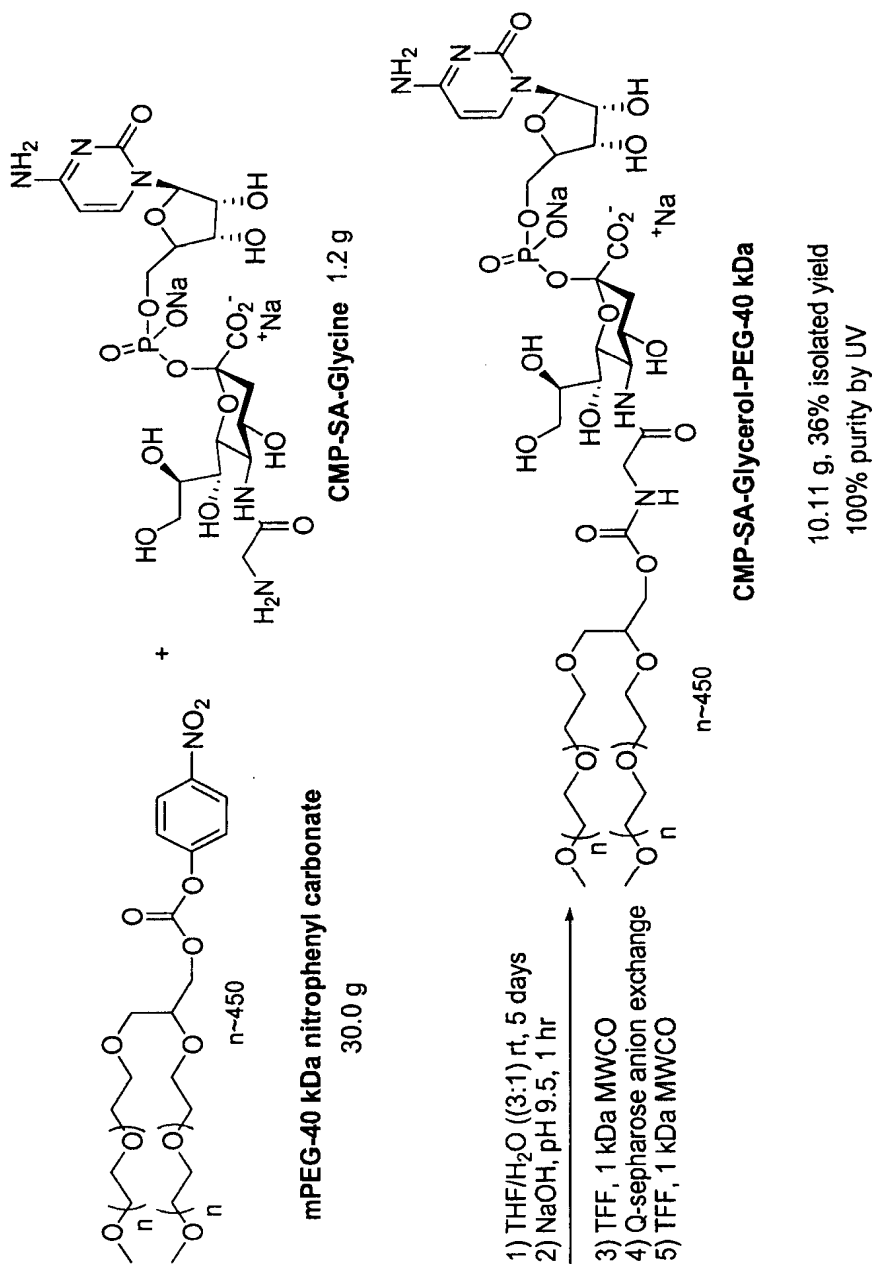
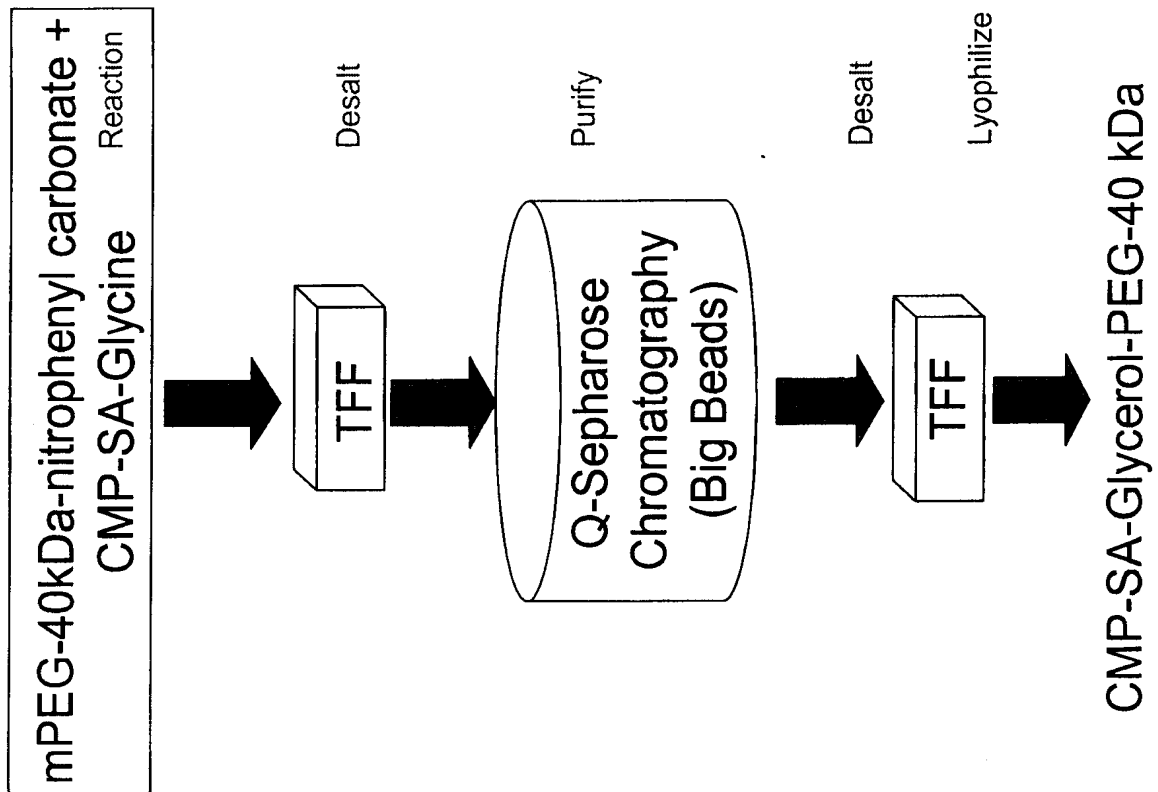


FIGURE 2

**CMP-SA-Glycerol-PEG-40kDa Reaction Conditions**

Reaction Parameters	
CMP-SA-Gly (Salt Form)	2.3 mol. eq. (1.2 g) Sodium Salt
mPEG-40kDa-nitrophenyl carbonate (NOF)	1 mol. eq. (30 g)
Solvents	THF:H <sub>2</sub> O (3:1)
pH	7.5 - 8
Temperature	20 °C
Reaction Time	5 days
Purified yield	36% 10.11 g
Purity (UV 274 nm vs. CMP-SA-Glycine)	100%

## CMP-SA-Glycerol-PEG-40kDa Purification Process



## Q-Sepharose Purification of CMP-SA-Glycerol-PEG-40 kDa

Q-Sepharose Big Beads (6L, 18 x 23 cm)

Bicarbonate Form of Resin

Mobile Phase A: Water

Mobile Phase B: 1.0 N NaCl

UV: 274 nm

Load: Approximately 15 g of CMP-SA-Glycerol-PEG-40 kDa Reaction

Mixture (30 g) after TFF (Conductivity: 0.53 mS)

Load Rate: 60 mL/min

Elution:

1.67 CV Mobile Phase A

2 CV gradient from 10% to 80% Mobile Phase B at 125 mL/min.

Elution Pool was desalted by TFF Millipore 1 kDa Pellicon 2 "MINI" (2 x PLAC 1 kDa Regenerated Cellulose Membrane; Screen Type V; 0.1m<sup>2</sup>).

Desalted product was Freeze-dried.

FIGURE 4

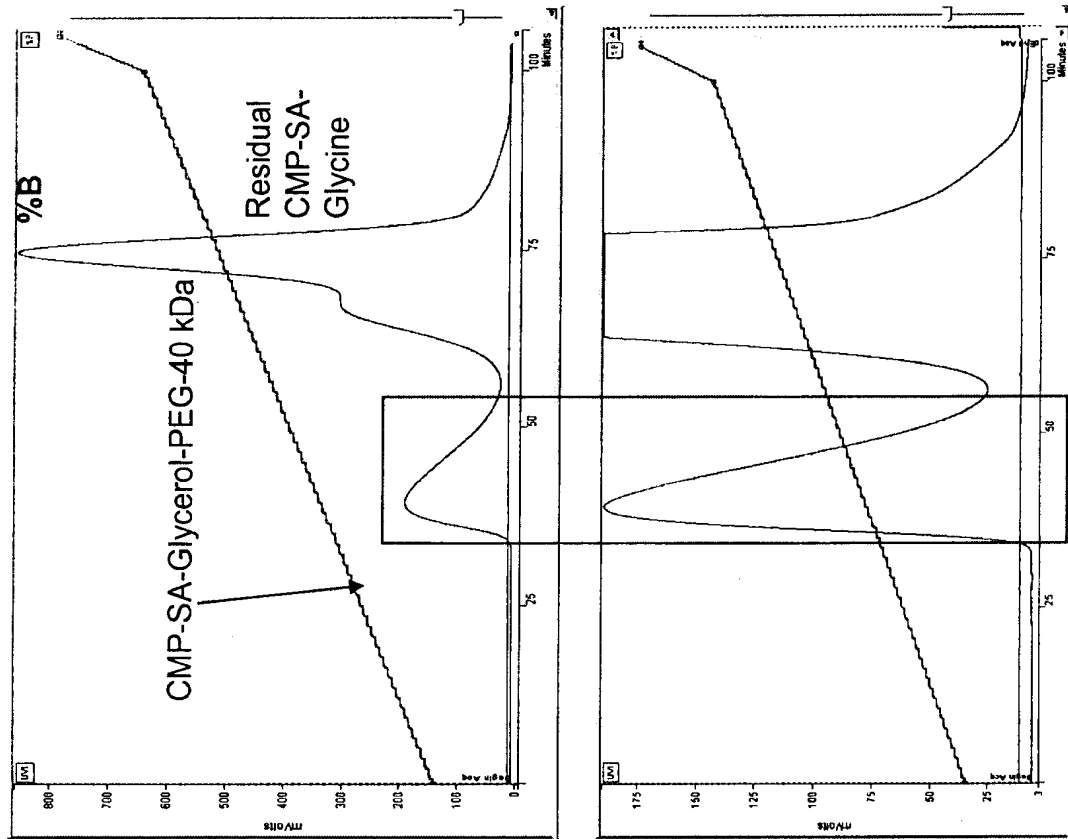


FIGURE 5

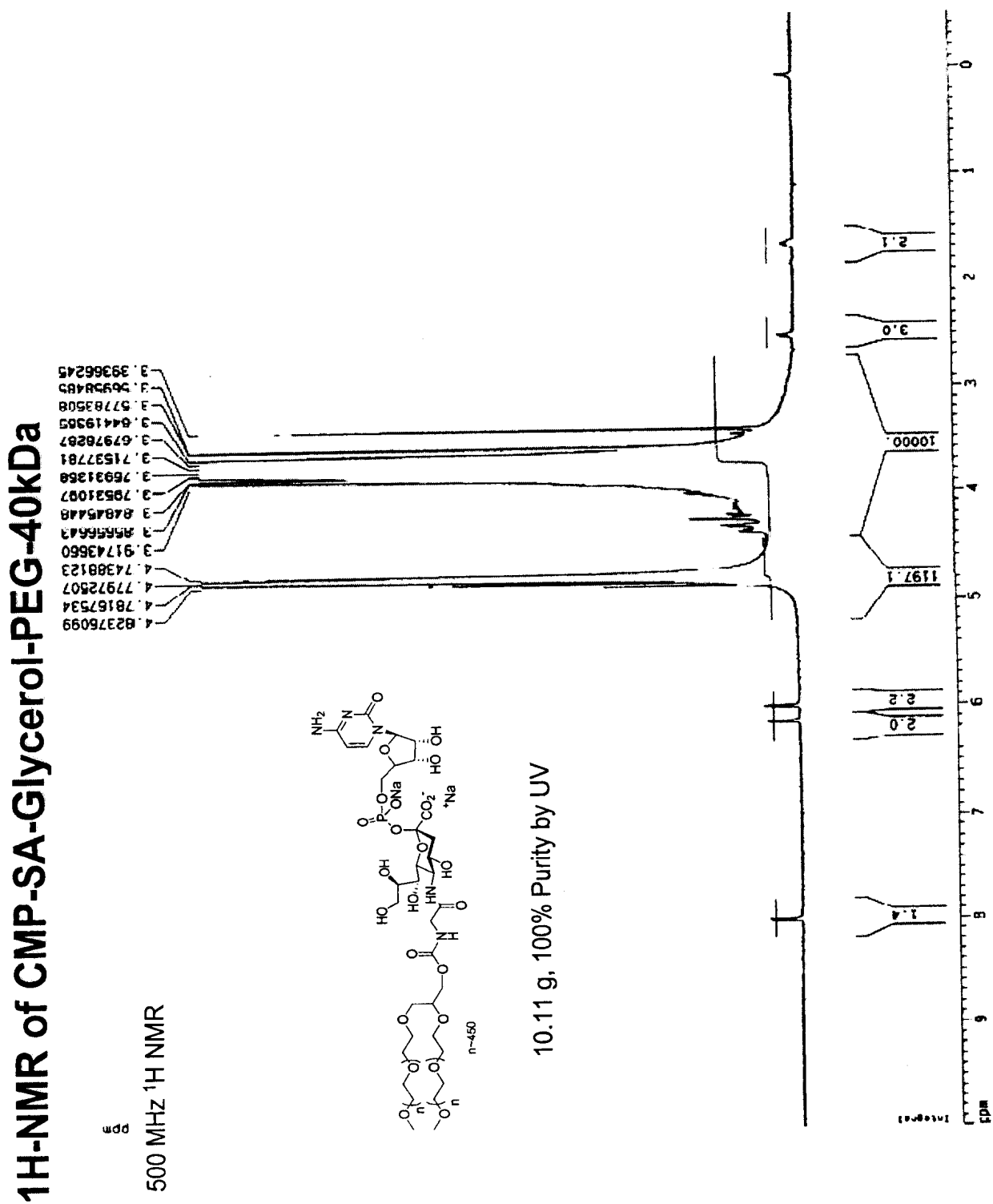


FIGURE 6A

Protein	Organism	EC#	GenBank / GenPept		SwissProt	PDB / 3D
At1g08280	<i>Arabidopsis thaliana</i>	n.d.	AC011438 BT004583 NC_003070	AAF18241.1 AAO42829.1 NP_172305.1	Q84W00 Q9SGD2	
At1g08660/F22O13.14	<i>Arabidopsis thaliana</i>	n.d.	AC003981 AY064135 AY124807 NC_003070 NM_180609	AAF99778.1 AAL36042.1 AAM70516.1 NP_172342.1 NP_850940.1	Q8VZJ0 Q9FRR9	
At3g48820/T21J18_90	<i>Arabidopsis thaliana</i>	n.d.	AY080589 AY133816 AL132963 NM_114741	AAL85966.1 AAM91750.1 CAB87910.1 NP_190451.1	Q8RY00 Q9M301	
$\alpha$ -2,3-sialyltransferase (ST3Gal-IV)	<i>Bos taurus</i>	n.d.	AJ584673	CAE48298.1		
$\alpha$ -2,3-sialyltransferase (ST3Gal-V)	<i>Bos taurus</i>	n.d.	AJ585768	CAE51392.1		
$\alpha$ -2,6-sialyltransferase (Siat7b)	<i>Bos taurus</i>	n.d.	AJ620651	CAF05850.1		
$\alpha$ -2,8-sialyltransferase (SIAT8A)	<i>Bos taurus</i>	2.4.99.8	AJ699418	CAG27880.1		
$\alpha$ -2,8-sialyltransferase (Siat8D)	<i>Bos taurus</i>	n.d.	AJ699421	CAG27883.1		
$\alpha$ -2,8-sialyltransferase ST8Sia-III (Siat8C)	<i>Bos taurus</i>	n.d.	AJ704563	CAG28696.1		
CMP $\alpha$ -2,6-sialyltransferase (ST6Gal I)	<i>Bos taurus</i>	2.4.99.1	Y15111 NM_177517	CAA75385.1 NP_803483.1	O18974	
sialyltransferase 8 (fragment)	<i>Bos taurus</i>	n.d.	AF450088	AAL47018.1	Q8WN13	
sialyltransferase ST3Gal-II (Siat4B)	<i>Bos taurus</i>	n.d.	AJ748841	CAG44450.1		
sialyltransferase ST3Gal-III (Siat6)	<i>Bos taurus</i>	n.d.	AJ748842	CAG44451.1		
sialyltransferase ST3Gal-VI (Siat10)	<i>Bos taurus</i>	n.d.	AJ748843	CAG44452.1		
ST3Gal I	<i>Bos taurus</i>	n.d.	AJ305086	CAC24698.1	Q9BEG4	
St6GalNAc-VI	<i>Bos taurus</i>	n.d.	AJ620949	CAF06586.1		
CDS4	<i>Branchiostoma floridae</i>	n.d.	AF391289	AAM18873.1	Q8T771	
polysialyltransferase (PST) (fragment) ST8Sia IV	<i>Cercopithecus aethiops</i>	2.4.99.-	AF210729	AAF17105.1	Q9TT09	
polysialyltransferase (STX) (fragment) ST8Sia II	<i>Cercopithecus aethiops</i>	2.4.99.-	AF210318	AAF17104.1	Q9TT10	
$\alpha$ -2,3-sialyltransferase ST3Gal I (Siat4)	<i>Ciona intestinalis</i>	n.d.	AJ626815	CAF25173.1		
$\alpha$ -2,3-sialyltransferase ST3Gal I (Siat4)	<i>Ciona savignyi</i>	n.d.	AJ626814	CAF25172.1		
$\alpha$ -2,8-polysialyltransferase ST8Sia IV	<i>Cricetulus griseus</i>	2.4.99.-	- Z46801	AAE28634 CAA86822.1	Q64690	
Gal $\beta$ -1,3/4-GlcNAc $\alpha$ -2,3-sialyltransferase ST3Gal I	<i>Cricetulus griseus</i>	n.d.	AY266675	AAP22942.1	Q80WL0	
Gal $\beta$ -1,3/4-GlcNAc $\alpha$ -2,3-sialyltransferase St3Gal II (fragment)	<i>Cricetulus griseus</i>	n.d.	AY266676	AAP22943.1	Q80WK9	
$\alpha$ -2,3-sialyltransferase ST3Gal I (Siat4)	<i>Danio rerio</i>	n.d.	AJ783740	CAH04017.1		
$\alpha$ -2,3-sialyltransferase ST3Gal II (Siat5)	<i>Danio rerio</i>	n.d.	AJ783741	CAH04018.1		

FIGURE 6B

Protein	Organism	EC#	GenBank / GenPept		SwissProt	PDB / 3D
$\alpha$ -2,3-sialyltransferase ST3Gal III (Siat6)	<i>Danio rerio</i>	n.d.	AJ626821	CAF25179.1		
$\alpha$ -2,3-sialyltransferase ST3Gal IV (Siat4c)	<i>Danio rerio</i>	n.d.	AJ744809	CAG32845.1		
$\alpha$ -2,3-sialyltransferase ST3Gal V-r (Siat5-related)	<i>Danio rerio</i>	n.d.	AJ783742	CAH04019.1		
$\alpha$ -2,6-sialyltransferase ST6Gal I (Siat1)	<i>Danio rerio</i>	n.d.	AJ744801	CAG32837.1		
$\alpha$ -2,6-sialyltransferase ST6GalNAc II (Siat7B)	<i>Danio rerio</i>	n.d.	AJ634459	CAG25680.1		
$\alpha$ -2,6-sialyltransferase ST6GalNAc V (Siat7E) (fragment)	<i>Danio rerio</i>	n.d.	AJ646874	CAG26703.1		
$\alpha$ -2,6-sialyltransferase ST6GalNAc VI (Siat7F) (fragment)	<i>Danio rerio</i>	n.d.	AJ646883	CAG26712.1		
$\alpha$ -2,8-sialyltransferase ST8Sia I (Siat 8A) (fragment)	<i>Danio rerio</i>	n.d.	AJ715535	CAG29374.1		
$\alpha$ -2,8-sialyltransferase ST8Sia III (Siat 8C) (fragment)	<i>Danio rerio</i>	n.d.	AJ715543	CAG29382.1		
$\alpha$ -2,8-sialyltransferase ST8Sia IV (Siat 8D) (fragment)	<i>Danio rerio</i>	n.d.	AJ715545	CAG29384.1		
$\alpha$ -2,8-sialyltransferase ST8Sia V (Siat 8E) (fragment)	<i>Danio rerio</i>	n.d.	AJ715546	CAG29385.1		
$\alpha$ -2,8-sialyltransferase ST8Sia VI (Siat 8F) (fragment)	<i>Danio rerio</i>	n.d.	AJ715551	CAG29390.1		
$\beta$ -galactosamide $\alpha$ -2,6-sialyltransferase II (ST6Gal II)	<i>Danio rerio</i>	n.d.	AJ627627	CAF29495.1		
N-glycan $\alpha$ -2,8-sialyltransferase	<i>Danio rerio</i>	n.d.	BC050483 AY055462 NM_153662	AAH50483.1 AAL17875.1 NP_705948.1	Q7ZU51 Q8QH83	
ST3Gal III-related (sial6r)	<i>Danio rerio</i>	n.d.	BC053179 AJ626820 NM_200355	AAH53179.1 CAF25178.1 NP_956649.1	Q7T3B9	
St3Gal-V	<i>Danio rerio</i>	n.d.	AJ619960	CAF04061.1		
st6GalNAc-VI	<i>Danio rerio</i>	n.d.	BC060932 AJ620947	AAH60932.1 CAF06584.1		
$\alpha$ -2,6-sialyltransferase (CG4871) ST6Gal I	<i>Drosophila melanogaster</i>	2.4.99.1	AE003465 AF218237 AF397532 AE003465 NM_079129 NM_166684	AAF47256.1 AAG13185.1 AAK92126.1 AAM70791.1 NP_523853.1 NP_726474.1	Q9GU23 Q9W121	
$\alpha$ -2,3-sialyltransferase (ST3Gal-VI)	<i>Gallus gallus</i>	n.d.	AJ585767 AJ627204	CAE51391.1 CAF25503.1		
$\alpha$ -2,3-sialyltransferase ST3Gal I	<i>Gallus gallus</i>	2.4.99.4	X80503 NM_205217	CAA56666.1 NP_990548.1	Q11200	
$\alpha$ -2,3-sialyltransferase ST3Gal IV (fragment)	<i>Gallus gallus</i>	2.4.99.-	AF035250	AAC14163.1	O73724	
$\alpha$ -2,3-sialyltransferase (ST3GAL-II)	<i>Gallus gallus</i>	n.d.	AJ585761	CAE51385.2		
$\alpha$ -2,6-sialyltransferase (Siat7b)	<i>Gallus gallus</i>	n.d.	AJ620653	CAF05852.1		
$\alpha$ -2,6-sialyltransferase ST6Gal I	<i>Gallus gallus</i>	2.4.99.1	X75558 NM_205241	CAA53235.1 NP_990572.1	Q92182	
$\alpha$ -2,6-sialyltransferase	<i>Gallus gallus</i>	2.4.99.3	-	AAE68028.1	Q92183	



FIGURE 6C

Protein	Organism	EC#	GenBank / GenPept	SwissProt	PDB / 3D
ST6GalNAc I			- X74946 NM_205240	AAE68029.1 CAA52902.1 NP_990571.1	
$\alpha$ -2,6-sialyltransferase ST6GalNAc II	<i>Gallus gallus</i>	2.4.99.-	X77775 NM_205233	AAE68030.1 CAA54813.1 NP_990564.1	Q92184
$\alpha$ -2,6-sialyltransferase ST6GalNAc III (SIAT7C) (fragment)	<i>Gallus gallus</i>	n.d.	AJ634455	CAG25677.1	
$\alpha$ -2,6-sialyltransferase ST6GalNAc V (SIAT7E) (fragment)	<i>Gallus gallus</i>	n.d.	AJ646877	CAG26706.1	
$\alpha$ -2,8-sialyltransferase (GD3 Synthase) ST8Sia I	<i>Gallus gallus</i>	2.4.99.-	U73176	AAC28888.1	P79783
$\alpha$ -2,8-sialyltransferase (SIAT8B)	<i>Gallus gallus</i>	n.d.	AJ699419	CAG27881.1	
$\alpha$ -2,8-sialyltransferase (SIAT8C)	<i>Gallus gallus</i>	n.d.	AJ699420	CAG27882.1	
$\alpha$ -2,8-sialyltransferase (SIAT8F)	<i>Gallus gallus</i>	n.d.	AJ699424	CAG27886.1	
$\alpha$ -2,8-sialyltransferase ST8Sia-V (SIAT8C)	<i>Gallus gallus</i>	n.d.	AJ704564	CAG28697.1	
$\beta$ -galactosamide $\alpha$ -2,6- sialyltransferase II (ST6Gal II)	<i>Gallus gallus</i>	n.d.	AJ627629	CAF29497.1	
GM3 synthase (SIAT9)	<i>Gallus gallus</i>	2.4.99.9	AY515255	AAS83519.1	
polysialyltransferase ST8Sia IV	<i>Gallus gallus</i>	2.4.99.-	AF008194	AAB95120.1	O42399
$\alpha$ -2,3-sialyltransferase ST3Gal I	<i>Homo sapiens</i>	2.4.99.4	L29555 AF059321 L13972 AF155238 AF186191 BC018357 NM_003033 NM_173344	AAA36612.1 AAC17874.1 AAC37574.1 AAD39238.1 AAG29876.1 AAH18357.1 NP_003024.1 NP_775479.1	Q11201 O60677 Q9UN51
$\alpha$ -2,3-sialyltransferase ST3Gal II	<i>Homo sapiens</i>	2.4.99.4	U63090 BC036777 X96667 NM_006927	AAB40389.1 AAH36777.1 CAA65447.1 NP_008858.1	Q16842 O00654
$\alpha$ -2,3-sialyltransferase ST3Gal III (SiaT6)	<i>Homo sapiens</i>	2.4.99.6	L23768 BC050380 AF425851 AF425852 AF425853 AF425854 AF425855 AF425856 AF425857 AF425858 AF425859 AF425860 AF425861 AF425862 AF425863 AF425864 AF425865 AF425866 AF425867 AY167992 AY167993 AY167994	AAA35778.1 AAH50380.1 AAO13859.1 AAO13860.1 AAO13861.1 AAO13862.1 AAO13863.1 AAO13864.1 AAO13865.1 AAO13866.1 AAO13867.1 AAO13868.1 AAO13869.1 AAO13870.1 AAO13871.1 AAO13872.1 AAO13873.1 AAO13874.1 AAO13875.1 AAO38806.1 AAO38807.1 AAO38808.1	Q11203 Q86UR6 Q86UR7 Q86UR8 Q86UR9 Q86US0 Q86US1 Q86US2 Q8IX43 Q8IX44 Q8IX45 Q8IX46 Q8IX47 Q8IX48 Q8IX49 Q8IX50 Q8IX51 Q8IX52 Q8IX53 Q8IX54 Q8IX55 Q8IX56

FIGURE 6D

Protein	Organism	EC#	GenBank / GenPept	SwissProt	PDB / 3D
			AY167995 AAO38809.1 AY167996 AAO38810.1 AY167997 AAO38811.1 AY167998 AAO38812.1 NM_006279 NP_006270.1 NM_174964 NP_777624.1 NM_174965 NP_777625.1 NM_174966 NP_777626.1 NM_174967 NP_777627.1 NM_174969 NP_777629.1 NM_174970 NP_777630.1 NM_174972 NP_777632.1	Q8IX57 Q8IX58	
$\alpha$ -2,3-sialyltransferase ST3Gal IV	<i>Homo sapiens</i>	2.4.99.-	L23767 AAA16460.1 AF035249 AAC14162.1 BC010645 AAH10645.1 AY040826 AAK93790.1 AF516602 AAM66431.1 AF516603 AAM66432.1 AF516604 AAM66433.1 AF525084 AAM81378.1 X74570 CAA52662.1 CR456858 CAG33139.1 NM_006278 NP_006269.1	Q11206 Q60497 Q96QQ9 Q8N6A6 Q8N6A7 Q8NFD3 Q8NFG7	
$\alpha$ -2,3-sialyltransferase ST3Gal VI	<i>Homo sapiens</i>	2.4.99.4	AF119391 AAD39131.1 BC023312 AAH23312.1 AB022918 BAA77609.1 AX877828 CAE89895.1 AX886023 CAF00161.1 NM_006100 NP_006091.1	Q9Y274	
$\alpha$ -2,6-sialyltransferase (ST6Gal II ; KIAA1877)	<i>Homo sapiens</i>	n.d.	BC008680 AAH08680.1 AB058780 BAB47506.1 AB059555 BAC24793.1 AJ512141 CAD54408.1 AX795193 CAE48260.1 AX795193 CAE48261.1 NM_032528 NP_115917.1	Q86Y44 Q8IUG7 Q96HE4 Q96JF0	
$\alpha$ -2,6-sialyltransferase (ST6GALNAC III)	<i>Homo sapiens</i>	n.d.	BC059363 AAH59363.1 AY358540 AAQ88904.1 AK091215 BAC03611.1 AJ507291 CAD45371.1 NM_152996 NP_694541.1	Q8N259 Q8NDV1	
$\alpha$ -2,6-sialyltransferase (ST6GalNAc V)	<i>Homo sapiens</i>	n.d.	BC001201 AAH01201.1 AK056241 BAB71127.1 AL035409 CAB72344.1 AJ507292 CAD45372.1 NM_030965 NP_112227.1	Q9BVH7	
$\alpha$ -2,6-sialyltransferase (SThM) ST6GalNAc II	<i>Homo sapiens</i>	2.4.99.-	U14550 AAA52228.1 BC040455 AAH40455.1 AJ251053 CAB61434.1 NM_006456 NP_006447.1	Q9UJ37 Q12971	
$\alpha$ -2,6-sialyltransferase ST6Gal I	<i>Homo sapiens</i>	2.4.99.1	BC031476 AAH31476.1 BC040009 AAH40009.1 A17362 CAA01327.1 A23699 CAA01686.1 X17247 CAA35111.1 X54363 CAA38246.1 X62822 CAA44634.1 NM_003032 NP_003023.1 NM_173216 NP_775323.1	P15907	
$\alpha$ -2,6-sialyltransferase ST6GalNAc I	<i>Homo sapiens</i>	2.4.99.3	BC022462 AAH22462.1 AY096001 AAM22800.1 AY358918 AAQ89277.1 AK000113 BAA90953.1 Y11339 CAA72179.2	Q8TBJ6 Q9NSC7 Q9NXQ7	

FIGURE 6E

Protein	Organism	EC#	GenBank / GenPept		SwissProt	PDB / 3D
			NM_018414	NP_060884.1		
$\alpha$ -2,8-polysialyltransferase ST8Sia IV	<i>Homo sapiens</i>	2.4.99.-	L41680 BC027866 BC053657 NM_005668	AAC41775.1 AAH27866.1 AAH53657.1 NP_005659.1	Q8N1F4 Q92187 Q92693	
$\alpha$ -2,8-sialyltransferase (GD3 synthase) ST8Sia I	<i>Homo sapiens</i>	2.4.99.8	L32867 L43494 BC046158 - AY569975 D26360 X77922 NM_003034	AAA62366.1 AAC37586.1 AAH46158.1 AAQ53140.1 AAS75783.1 BAA05391.1 CAA54891.1 NP_003025.1	Q86X71 Q92185 Q93064	
$\alpha$ -2,8-sialyltransferase ST8Sia II	<i>Homo sapiens</i>	2.4.99.-	L29556 U82762 U33551 BC069584 NM_006011	AAA36613.1 AAB51242.1 AAC24458.1 AAH69584.1 NP_006002.1	Q92186 Q92470 Q92746	
$\alpha$ -2,8-sialyltransferase ST8Sia III	<i>Homo sapiens</i>	2.4.99.-	AF004668 AF003092 NM_015879	AAB87642.1 AAC15901.2 NP_056963.1	O43173 Q9NS41	
$\alpha$ -2,8-sialyltransferase ST8Sia V	<i>Homo sapiens</i>	2.4.99.-	U91641 CR457037 NM_013305	AAC51727.1 CAG33318.1 NP_037437.1	O15466	
ENSP00000020221 (fragment)		n.d.	AC023295	-		
lactosylceramide $\alpha$ -2,3-sialyltransferase (ST3Gal V)	<i>Homo sapiens</i>	2.4.99.9	AF105026 AF119415 BC065936 AY152815 AAP65066 AY359105 AB018356 AX876536 NM_003896	AAD14634.1 AAF66146.1 AAH65936.1 AAO16866.1 AAP65066.1 AAQ89463.1 BAA33950.1 CAE89320.1 NP_003887.2	Q9UNP4 O94902	
N-acetylgalactosaminide $\alpha$ -2,6-sialyltransferase (ST6GalNAc VI)	<i>Homo sapiens</i>	2.4.99.-	BC006564 BC007802 BC016299 AY358672 AB035173 AK023900 AJ507293 AX880950 CR457318 NM_013443	AAH06564.1 AAH07802.1 AAH16299.1 AAQ89035.1 BAA87035.1 BAB14715.1 CAD45373.1 CAE91145.1 CAG33599.1 NP_038471.2	Q969X2 Q9H8A2 Q9ULB8	
N-acetylgalactosaminide $\alpha$ -2,6-sialyltransferase IV (ST6GalNAc IV)	<i>Homo sapiens</i>	2.4.99.-	AF127142 BC036705 - AB035172 AK000600 Y17461 AJ271734 AX061620 AX068265 AX969252 NM_014403 NM_175039	AAF00102.1 AAH36705.1 AAP63349.1 BAA87034.1 BAA91281.1 CAB44354.1 CAC07404.1 CAC24981.1 CAC27250.1 CAF14360.1 NP_055218.3 NP_778204.1	Q9H4F1 Q9NWU6 Q9UKU1 Q9ULB9 Q9Y3G3 Q9Y3G4	
ST8SIA-VI (fragment)	<i>Homo sapiens</i>	n.d.	AJ621583 XM_291725	CAF21722.1 XP_291725.2		
unnamed protein product	<i>Homo sapiens</i>	n.d.	AK021929 AX881696	BAB13940.1 CAE91353.1	Q9HAA9	
Gal $\beta$ -1,3/4-GlcNAc $\alpha$ -	<i>Mesocricetus</i>	2.4.99.6	AJ245699	CAB53394.1	Q9QXF6	

FIGURE 6F

Protein	Organism	EC#	GenBank / GenPept		SwissProt	PDB / 3D
2,3-sialyltransferase (ST3Gal III)	<i>auratus</i>					
Gal $\beta$ 1,3/4-GlcNAc $\alpha$ -2,3-sialyltransferase (ST3Gal IV)	<i>Mesocricetus auratus</i>	2.4.99.6	AJ245700	CAB53395.1	Q9QXF5	
GD3 synthase (fragment) ST8Sia I	<i>Mesocricetus auratus</i>	n.d.	AF141657	AAD33879.1	Q9WUL1	
polysialyltransferase (ST8Sia IV)	<i>Mesocricetus auratus</i>	2.4.99.-	AJ245701	CAB53396.1	Q9QXF4	
$\alpha$ -2,3-sialyltransferase ST3Gal I	<i>St3gal1</i> <i>Mus musculus</i>	2.4.99.4	AF214028 AK031344 AK078469 X73523 NM_009177	AAF60973.1 BAC27356.1 BAC37290.1 CAA51919.1 NP_033203.1	P54751 Q11202 Q9JL30	
$\alpha$ -2,3-sialyltransferase ST3Gal II	<i>St3gal2</i> <i>Mus musculus</i>	2.4.99.4	BC015264 BC066064 AK034554 AK034863 AK053827 X76989 NM_009179 NM_178048	AAH15264.1 AAH66064.1 BAC28752.1 BAC28859.1 BAC35543.1 CAA54294.1 NP_033205.1 NP_835149.1	Q11204 Q8BPL0 Q8BSA0 Q8BSE9 Q91WH6	
$\alpha$ -2,3-sialyltransferase ST3Gal III	<i>St3gal3</i> <i>Mus musculus</i>	2.4.99.-	BC006710 AK005053 AK013016 X84234 NM_009176	AAH06710.1 BAB23779.1 BAB28598.1 CAA59013.1 NP_033202.2	P97325 Q922X5 Q9CZ48 Q9DBB6	
$\alpha$ -2,3-sialyltransferase ST3Gal IV	<i>St3gal4</i> <i>Mus musculus</i>	2.4.99.4	BC011121 BC050773 D28941 AK008543 AB061305 X95809 NM_009178	AAH11121.1 AAH50773.1 BAA06068.1 BAB25732.1 BAB47508.1 CAA65076.1 NP_033204.2	P97354 Q61325 Q91Y74 Q921R5 Q9CVE8	
$\alpha$ -2,3-sialyltransferase ST3Gal VI	<i>St3gal6</i> <i>Mus musculus</i>	2.4.99.4	AF119390 BC052338 AB063326 AK033562 AK041173 NM_018784	AAD39130.1 AAH52338.1 BAB79494.1 BAC28360.1 BAC30851.1 NP_061254	Q80UR7 Q8BLV1 Q8VIB3 Q9WVG2	
$\alpha$ -2,6-sialyltransferase ST6GalNAc II	<i>St6galnac2</i> <i>Mus musculus</i>	2.4.99.-	NM_009180 BC010208 AB027198 AK004613 X93999 X94000 NM_009180	6677963 AAH10208.1 BAB00637.1 BAB23410.1 CAA63821.1 CAA63822.1 NP_033206.2	P70277 Q9DC24 Q9JIM5	
$\alpha$ -2,6-sialyltransferase ST6Gal I	<i>St6gal1</i> <i>Mus musculus</i>	2.4.99.1	- BC027833 D16106 AK034768 AK084124 NM_145933	AAE68031.1 AAH27833.1 BAA03680.1 BAC28828.1 BAC39120.1 NP_666045.1	Q64685 Q8BM62 Q8K1L1	
$\alpha$ -2,6-sialyltransferase ST6Gal II	<i>St6gal2</i> <i>Mus musculus</i>	n.d.	AK082566 AB095093 AK129462 NM_172829	BAC38534.1 BAC87752.1 BAC98272.1 NP_766417.1	Q8BUU4	
$\alpha$ -2,6-sialyltransferase ST6GalNAc I	<i>St6galnac1</i> <i>Mus musculus</i>	2.4.99.3	Y11274 NM_011371	CAA72137.1 NP_035501.1	Q9QZ39 Q9JJP5	
$\alpha$ -2,6-sialyltransferase ST6GalNAc III	<i>St6galnac3</i> <i>Mus musculus</i>	n.d.	BC058387 AK034804 Y11342 Y11343	AAH58387.1 BAC28836.1 CAA72181.2 CAB95031.1	Q9WUV2 Q9JHP5	

FIGURE 6G

Protein	Organism		EC#	GenBank / GenPept		SwissProt	PDB / 3D
				NM_011372	NP_035502		
$\alpha$ -2,6-sialyltransferase ST6GalNAc IV	<i>St6galnac4</i>	<i>Mus musculus</i>	2.4.99.7	BC056451 AK085730 AJ007310 Y15779 Y15780 Y19055 Y19057 NM_011373	AAH56451.1 BAC39523.1 CAA07446.1 CAB43507.1 <b>CAB43514.1</b> CAB93946.1 CAB93948.1 NP_035503.1	Q8C3J2 Q9JHP2 Q9R2B6 O88725 Q9JHP0 Q9QUP9 Q9R2B5	
$\alpha$ -2,8-sialyltransferase (GD3 synthase) ST8Sia I	<i>St8sia1</i>	<i>Mus musculus</i>	2.4.99.8	L38677 BC024821 AK046188 AK052444 X84235 AJ401102 NM_011374	AAA91869.1 <b>AAH24821.1</b> BAC32625.1 BAC34994.1 CAA59014.1 CAC20706.1 NP_035504.1	Q64468 Q64687 Q8BL76 Q8BWI0 Q8K1C1 Q9EPK0	
$\alpha$ -2,8-sialyltransferase (ST8Sia VI)	<i>St8sia6</i>	<i>Mus musculus</i>	n.d.	AB059554 AK085105 NM_145838	<b>BAC01265.1</b> BAC39367.1 NP_665837.1	Q8BI43 Q8K4T1	
$\alpha$ -2,8-sialyltransferase ST8Sia II	<i>St8sia2</i>	<i>Mus musculus</i>	2.4.99.-	X83562 X99646 X99647 X99648 X99649 X99650 X99651 NM_009181	CAA58548.1 CAA67965.1 CAA67965.1 CAA67965.1 CAA67965.1 CAA67965.1 CAA67965.1 NP_033207.1	<b>O35696</b>	
$\alpha$ -2,8-sialyltransferase ST8Sia IV	<i>St8sia4</i>	<i>Mus musculus</i>	2.4.99.8	BC060112 AK003690 AK041723 AJ223956 X86000 Y09484 NM_009183	AAH60112.1 BAB22941.1 BAC31044.1 <b>CAA11685.1</b> CAA59992.1 CAA70692.1 NP_033209.1	Q64692 Q8BY70	
$\alpha$ -2,8-sialyltransferase ST8Sia V	<i>St8sia5</i>	<i>Mus musculus</i>	2.4.99.-	BC034855 AK078670 X98014 X98014 X98014 NM_013666 NM_153124 NM_177416	AAH34855.1 BAC37354.1 <b>CAA66642.1</b> CAA66643.1 CAA66644.1 NP_038694.1 NP_694764.1 NP_803135.1	P70126 P70127 P70128 Q8BJW0 Q8JZQ3	
$\alpha$ -2,8-sialyltransferase ST8Sia III	<i>St8sia3</i>	<i>Mus musculus</i>	2.4.99.-	BC075645 AK015874 X80502 NM_009182	AAH75645.1 BAB30012.1 CAA56665.1 NP_033208.1	<b>Q64689</b> Q9CUJ6	
GD1 synthase (ST6GalNAc V)	<i>St6galnac5</i>	<i>Mus musculus</i>	n.d.	BC055737 AB030836 AB028840 AK034387 AK038434 AK042683 NM_012028	<b>AAH55737.1</b> BAA85747.1 BAA89292.1 BAC28693.1 BAC29997.1 BAC31331.1 NP_036158.2	Q8CAM7 Q8CBX1 Q9QYJ1 Q9R0K6	
GM3 synthase ( $\alpha$ -2,3- sialyltransferase) ST3Gal V	<i>St3gal5</i>	<i>Mus musculus</i>	2.4.99.9	AF119416 - AB018048 AB013302 AK012961 Y15003 NM_011375	<b>AAF66147.1</b> AAP65063.1 BAA33491.1 BAA76467.1 BAB28571.1 CAA75235.1 NP_035505.1	O88829 Q9CZ65 Q9QWF9	
N- acetylgalactosaminide $\alpha$ -2,6-sialyltransferase (ST6GalNAc VI)	<i>St6galnac6</i>	<i>Mus musculus</i>	2.4.99.-	BC036985 AB035174 AB035123 AK030648	<b>AAH36985.1</b> BAA87036.1 BAA95940.1 BAC27064.1	Q8CDC3 Q8JZW3 Q9JM95 Q9R0G9	

FIGURE 6H

Protein	Organism	EC#	GenBank / GenPept		SwissProt	PDB / 3D
			NM_016973	NP_058669.1		
M138L	<i>Myxoma virus</i>	n.d.	U46578 AF170726 NC_001132	AAD00069.1 AAE61323.1 AAE61326.1 AAF15026.1 NP_051852.1		
$\alpha$ -2,3-sialyltransferase (St3Gal-I)	<i>Oncorhynchus mykiss</i>	n.d.	AJ585760	CAE51384.1		
$\alpha$ -2,6-sialyltransferase (Siat1)	<i>Oncorhynchus mykiss</i>	n.d.	AJ620649	CAF05848.1		
$\alpha$ -2,8-polysialyltransferase IV (ST8Sia IV)	<i>Oncorhynchus mykiss</i>	n.d.	AB094402	BAC77411.1	Q7T2X5	
GalNAc $\alpha$ -2,6-sialyltransferase (RtST6GalNAc)	<i>Oncorhynchus mykiss</i>	n.d.	AB097943	BAC77520.1	Q7T2X4	
$\alpha$ -2,3-sialyltransferase ST3Gal IV	<i>Oryctolagus cuniculus</i>	2.4.99.-	AF121967	AAF28871.1	Q9N257	
OJ1217_F02.7	<i>Oryza sativa (japonica cultivar-group)</i>	n.d.	AP004084	BAD07616.1		
OSJNBa0043L24.2 or OSJNBb0002J11.9	<i>Oryza sativa (japonica cultivar-group)</i>	n.d.	AL731626 AL662969	CAD41185.1 CAE04714.1		
P0683f02.18 or P0489B03.1	<i>Oryza sativa (japonica cultivar-group)</i>	n.d.	AP003289 AP003794	BAB63715.1 BAB90552.1		
$\alpha$ -2,6-sialyltransferase ST6GalNAc V (Siat7E) (fragment)	<i>Oryzias latipes</i>	n.d.	AJ646876	CAG26705.1		
$\alpha$ -2,3-sialyltransferase ST3Gal I (Siat4)	<i>Pan troglodytes</i>	n.d.	AJ744803	CAG32839.1		
$\alpha$ -2,3-sialyltransferase ST3Gal II (Siat5)	<i>Pan troglodytes</i>	n.d.	AJ744804	CAG32840.1		
$\alpha$ -2,3-sialyltransferase ST3Gal III (Siat6)	<i>Pan troglodytes</i>	n.d.	AJ626819	CAF25177.1		
$\alpha$ -2,3-sialyltransferase ST3Gal IV (Siat4c)	<i>Pan troglodytes</i>	n.d.	AJ626824	CAF25182.1		
$\alpha$ -2,3-sialyltransferase ST3Gal VI (Siat10)	<i>Pan troglodytes</i>	n.d.	AJ744808	CAG32844.1		
$\alpha$ -2,6-sialyltransferase (Sia7A)	<i>Pan troglodytes</i>	n.d.	AJ748740	CAG38615.1		
$\alpha$ -2,6-sialyltransferase (Sia7B)	<i>Pan troglodytes</i>	n.d.	AJ748741	CAG38616.1		
$\alpha$ -2,6-sialyltransferase ST6GalNAc III (Siat7C)	<i>Pan troglodytes</i>	n.d.	AJ634454	CAG25676.1		
$\alpha$ -2,6-sialyltransferase ST6GalNAc IV (Siat7D) (fragment)	<i>Pan troglodytes</i>	n.d.	AJ646870	CAG26699.1		
$\alpha$ -2,6-sialyltransferase ST6GalNAc V (Siat7E)	<i>Pan troglodytes</i>	n.d.	AJ646875	CAG26704.1		
$\alpha$ -2,6-sialyltransferase ST6GalNAc VI (Siat7F) (fragment)	<i>Pan troglodytes</i>	n.d.	AJ646882	CAG26711.1		
$\alpha$ -2,8-sialyltransferase 8A (Siat8A)	<i>Pan troglodytes</i>	2.4.99.8	AJ697658	CAG26896.1		
$\alpha$ -2,8-sialyltransferase 8B (Siat8B)	<i>Pan troglodytes</i>	n.d.	AJ697659	CAG26897.1		
$\alpha$ -2,8-sialyltransferase 8C (Siat8C)	<i>Pan troglodytes</i>	n.d.	AJ697660	CAG26898.1		
$\alpha$ -2,8-sialyltransferase 8D (Siat8D)	<i>Pan troglodytes</i>	n.d.	AJ697661	CAG26899.1		
$\alpha$ -2,8-sialyltransferase	<i>Pan troglodytes</i>	n.d.	AJ697662	CAG26900.1		

FIGURE 61

Protein	Organism	EC#	GenBank / GenPept		SwissProt	PDB / 3D
8E (Siat8E)						
$\alpha$ -2,8-sialyltransferase 8F (Siat8F)	<i>Pan troglodytes</i>	n.d.	AJ697663	CAG26901.1		
$\beta$ -galactosamide $\alpha$ -2,6-sialyltransferase I (ST6Gal I; Siat1)	<i>Pan troglodytes</i>	2.4.99.1	AJ627624	CAF29492.1		
$\beta$ -galactosamide $\alpha$ -2,6-sialyltransferase II (ST6Gal II)	<i>Pan troglodytes</i>	n.d.	AJ627625	CAF29493.1		
GM3 synthase ST3Gal V (Siat9)	<i>Pan troglodytes</i>	n.d.	AJ744807	CAG32843.1		
S138L	<i>Rabbit fibroma virus Kasza</i>	n.d.	NC_001266	NP_052025		
$\alpha$ -2,3-sialyltransferase ST3Gal III	<i>Rattus norvegicus</i>	2.4.99.6	M97754 NM_031697	AAA42146.1 NP_113885.1	Q02734	
$\alpha$ -2,3-sialyltransferase ST3Gal IV (Siat4c)	<i>Rattus norvegicus</i>	n.d.	AJ626825	CAF25183.1		
$\alpha$ -2,3-sialyltransferase ST3Gal VI	<i>Rattus norvegicus</i>	n.d.	AJ626743	CAF25053.1		
$\alpha$ -2,6-sialyltransferase ST3Gal II	<i>Rattus norvegicus</i>	2.4.99.-	X76988 NM_031695	CAA54293.1 NP_113883.1	Q11205	
$\alpha$ -2,6-sialyltransferase ST6Gal I	<i>Rattus norvegicus</i>	2.4.99.1	M18769 M83143	AAA41196.1 AAB07233.1	P13721	
$\alpha$ -2,6-sialyltransferase ST6GalNAc I (Siat7A)	<i>Rattus norvegicus</i>	n.d.	AJ634458	CAG25684.1		
$\alpha$ -2,6-sialyltransferase ST6GalNAc II (Siat7B)	<i>Rattus norvegicus</i>	n.d.	AJ634457	CAG25679.1		
$\alpha$ -2,6-sialyltransferase ST6GalNAc III	<i>Rattus norvegicus</i>	2.4.99.-	L29554 BC072501 NM_019123	AAC42086.1 AAH72501.1 NP_061996.1	Q64686	
$\alpha$ -2,6-sialyltransferase ST6GalNAc IV (Siat7D) (fragment)	<i>Rattus norvegicus</i>	n.d.	AJ646871	CAG26700.1		
$\alpha$ -2,6-sialyltransferase ST6GalNAc V (Siat7E)	<i>Rattus norvegicus</i>	n.d.	AJ646872	CAG26701.1		
$\alpha$ -2,6-sialyltransferase ST6GalNAc VI (Siat7F) (fragment)	<i>Rattus norvegicus</i>	n.d.	AJ646881	CAG26710.1		
$\alpha$ -2,8-sialyltransferase (GD3 synthase) ST8Sia I	<i>Rattus norvegicus</i>	2.4.99.-	U53883 D45255	AAC27541.1 BAA08213.1	P70554 P97713	
$\alpha$ -2,8-sialyltransferase (SIAT8E)	<i>Rattus norvegicus</i>	n.d.	AJ699422	CAG27884.1		
$\alpha$ -2,8-sialyltransferase (SIAT8F)	<i>Rattus norvegicus</i>	n.d.	AJ699423	CAG27885.1		
$\alpha$ -2,8-sialyltransferase ST8Sia II	<i>Rattus norvegicus</i>	2.4.99.-	L13445 NM_057156	AAA42147.1 NP_476497.1	Q07977 Q64688	
$\alpha$ -2,8-sialyltransferase ST8Sia III	<i>Rattus norvegicus</i>	2.4.99.-	U55938 NM_013029	AAB50061.1 NP_037161.1	P97877	
$\alpha$ -2,8-sialyltransferase ST8Sia IV	<i>Rattus norvegicus</i>	2.4.99.-	U90215	AAB49989.1	O08563	
$\beta$ -galactosamide $\alpha$ -2,6-sialyltransferase II (ST6Gal II)	<i>Rattus norvegicus</i>	n.d.	AJ627626	CAF29494.1		
GM3 synthase ST3Gal V	<i>Rattus norvegicus</i>	n.d.	AB018049 NM_031337	BAA33492.1 NP_112627.1	O88830	

FIGURE 6J

Protein	Organism	EC#	GenBank / GenPept		SwissProt	PDB / 3D
sialyltransferase ST3Gal-I (Siat4A)	<i>Rattus norvegicus</i>	n.d.	AJ748840	CAG44449.1		
$\alpha$ -2,3-sialyltransferase (ST3Gal-II)	<i>Silurana tropicalis</i>	n.d.	AJ585763	CAE51387.1		
$\alpha$ -2,6-sialyltransferase (Siat7b)	<i>Silurana tropicalis</i>	n.d.	AJ620650	CAF05849.1		
$\alpha$ -2,6-sialyltransferase (ST6galnac)	<i>Strongylocentrotus purpuratus</i>	n.d.	AJ699425	CAG27887.1		
$\alpha$ -2,3-sialyltransferase (ST3GAL-III)	<i>Sus scrofa</i>	n.d.	AJ585765	CAE51389.1		
$\alpha$ -2,3-sialyltransferase (ST3GAL-IV)	<i>Sus scrofa</i>	n.d.	AJ584674	CAE48299.1		
$\alpha$ -2,3-sialyltransferase ST3Gal I	<i>Sus scrofa</i>	2.4.99.4	M97753	AAA31125.1	Q02745	
$\alpha$ -2,6-sialyltransferase (fragment) ST6Gal I	<i>Sus scrofa</i>	2.4.99.1	AF136746	AAD33059.1	Q9XSG8	
$\beta$ -galactosamide $\alpha$ -2,6- sialyltransferase (ST6GalNAc-V)	<i>Sus scrofa</i>	n.d.	AJ620948	CAF06585.2		
sialyltransferase (fragment) ST6Gal I	<i>sus scrofa</i>	n.d.	AF041031	AAC15633.1	O62717	
ST6GALNAC-V	<i>Sus scrofa</i>	n.d.	AJ620948	CAF06585.1		
$\alpha$ -2,3-sialyltransferase (Siat5-r)	<i>Takifugu rubripes</i>	n.d.	AJ744805	CAG32841.1		
$\alpha$ -2,3-sialyltransferase ST3Gal I (Siat4)	<i>Takifugu rubripes</i>	n.d.	AJ626816	CAF25174.1		
$\alpha$ -2,3-sialyltransferase ST3Gal II (Siat5) (fragment)	<i>Takifugu rubripes</i>	n.d.	AJ626817	CAF25175.1		
$\alpha$ -2,3-sialyltransferase ST3Gal III (Siat6)	<i>Takifugu rubripes</i>	n.d.	AJ626818	CAF25176.1		
$\alpha$ -2,6-sialyltransferase ST6Gal I (Siat1)	<i>Takifugu rubripes</i>	n.d.	AJ744800	CAG32836.1		
$\alpha$ -2,6-sialyltransferase ST6GalNAc II (Siat7B)	<i>Takifugu rubripes</i>	n.d.	AJ634460	CAG25681.1		
$\alpha$ -2,6-sialyltransferase ST6GalNAc II B (Siat7B- related)	<i>Takifugu rubripes</i>	n.d.	AJ634461	CAG25682.1		
$\alpha$ -2,6-sialyltransferase ST6GalNAc III (Siat7C) (fragment)	<i>Takifugu rubripes</i>	n.d.	AJ634456	CAG25678.1		
$\alpha$ -2,6-sialyltransferase ST6GalNAc IV (siat7D) (fragment)	<i>Takifugu rubripes</i>	2.4.99.3	Y17466 AJ646869	CAB44338.1 CAG26698.1	Q9W6U6	
$\alpha$ -2,6-sialyltransferase ST6GalNAc V (Siat7E) (fragment)	<i>Takifugu rubripes</i>	n.d.	AJ646873	CAG26702.1		
$\alpha$ -2,6-sialyltransferase ST6GalNAc VI (Siat7F) (fragment)	<i>Takifugu rubripes</i>	n.d.	AJ646880	CAG26709.1		
$\alpha$ -2,8-sialyltransferase ST8Sia I (Siat 8A) (fragment)	<i>Takifugu rubripes</i>	n.d.	AJ715534	CAG29373.1		
$\alpha$ -2,8-sialyltransferase ST8Sia II (Siat 8B) (fragment)	<i>Takifugu rubripes</i>	n.d.	AJ715538	CAG29377.1		
$\alpha$ -2,8-sialyltransferase ST8Sia III (Siat 8C) (fragment)	<i>Takifugu rubripes</i>	n.d.	AJ715541	CAG29380.1		
$\alpha$ -2,8-sialyltransferase ST8Sia IIIr (Siat 8Cr)	<i>Takifugu rubripes</i>	n.d.	AJ715542	CAG29381.1		
$\alpha$ -2,8-sialyltransferase ST8Sia V (Siat 8E)	<i>Takifugu rubripes</i>	n.d.	AJ715547	CAG29386.1		



FIGURE 6K

Protein	Organism	EC#	GenBank / GenPept		SwissProt	PDB / 3D
(fragment)						
$\alpha$ -2,8-sialyltransferase ST8Sia VI (Siat 8F) (fragment)	<i>Takifugu rubripes</i>	n.d.	AJ715549	CAG29388.1		
$\alpha$ -2,8-sialyltransferase ST8Sia VIr (Siat 8Fr)	<i>Takifugu rubripes</i>	n.d.	AJ715550	CAG29389.1		
$\alpha$ -2,3-sialyltransferase (Siat5-r)	<i>Tetraodon nigroviridis</i>	n.d.	AJ744806	CAG32842.1		
$\alpha$ -2,3-sialyltransferase ST3Gal I (Siat4)	<i>Tetraodon nigroviridis</i>	n.d.	AJ744802	CAG32838.1		
$\alpha$ -2,3-sialyltransferase ST3Gal III (Siat6)	<i>Tetraodon nigroviridis</i>	n.d.	AJ626822	CAF25180.1		
$\alpha$ -2,6-sialyltransferase ST6GalNAc II (Siat7B)	<i>Tetraodon nigroviridis</i>	n.d.	AJ634462	CAG25683.1		
$\alpha$ -2,6-sialyltransferase ST6GalNAc V (Siat7E) (fragment)	<i>Tetraodon nigroviridis</i>	n.d.	AJ646879	CAG26708.1		
$\alpha$ -2,8-sialyltransferase ST8Sia I (Siat 8A) (fragment)	<i>Tetraodon nigroviridis</i>	n.d.	AJ715536	CAG29375.1		
$\alpha$ -2,8-sialyltransferase ST8Sia II (Siat 8B) (fragment)	<i>Tetraodon nigroviridis</i>	n.d.	AJ715537	CAG29376.1		
$\alpha$ -2,8-sialyltransferase ST8Sia III (Siat 8C) (fragment)	<i>Tetraodon nigroviridis</i>	n.d.	AJ715539	CAG29378.1		
$\alpha$ -2,8-sialyltransferase ST8Sia IIIr (Siat 8Cr) (fragment)	<i>Tetraodon nigroviridis</i>	n.d.	AJ715540	CAG29379.1		
$\alpha$ -2,8-sialyltransferase ST8Sia V (Siat 8E) (fragment)	<i>Tetraodon nigroviridis</i>	n.d.	AJ715548	CAG29387.1		
$\alpha$ -2,3-sialyltransferase (St3Gal-II)	<i>Xenopus laevis</i>	n.d.	AJ585762	CAE51386.1		
$\alpha$ -2,3-sialyltransferase (St3Gal-VI)	<i>Xenopus laevis</i>	n.d.	AJ585766	CAE51390.1		
$\alpha$ -2,3-sialyltransferase St3Gal-III (Siat6)	<i>Xenopus laevis</i>	n.d.	AJ585764 AJ626823	CAE51388.1 CAF25181.1		
$\alpha$ -2,8-polysialyltransferase	<i>Xenopus laevis</i>	2.4.99.-	AB007468	BAA32617.1	O93234	
$\alpha$ -2,8-sialyltransferase ST8Sia-I (Siat8A;GD3 synthase)	<i>Xenopus laevis</i>	n.d.	AY272056 AY272057 AJ704562	AAQ16162.1 AAQ16163.1 CAG28695.1		
Unknown (protein for MGC:81265)	<i>Xenopus laevis</i>	n.d.	BC068760	AAH68760.1		
$\alpha$ -2,3-sialyltransferase (3Gal-VI)	<i>Xenopus tropicalis</i>	n.d.	AJ626744	CAF25054.1		
$\alpha$ -2,3-sialyltransferase (Siat4c)	<i>Xenopus tropicalis</i>	n.d.	AJ622908	CAF22058.1		
$\alpha$ -2,6-sialyltransferase ST6GalNAc V (Siat7E) (fragment)	<i>Xenopus tropicalis</i>	n.d.	AJ646878	CAG26707.1		
$\alpha$ -2,8-sialyltransferase ST8Sia III (Siat 8C) (fragment)	<i>Xenopus tropicalis</i>	n.d.	AJ715544	CAG29383.1		
$\beta$ -galactosamide $\alpha$ -2,6-sialyltransferase II (ST6Gal II)	<i>Xenopus tropicalis</i>	n.d.	AJ627628	CAF29496.1		
sialyltransferase St8Sial	<i>Xenopus tropicalis</i>	n.d.	AY652775	AAT67042		
poly- $\alpha$ -2,8-sialosyl sialyltransferase (NeuS)	<i>Escherichia coli K1</i>	2.4.-.-	M76370 X60598	AAA24213.1 CAA43053.1	Q57269	
polysialyltransferase	<i>Escherichia coli K92</i>	2.4.-.-	M88479	AAA24215.1	Q47404	

FIGURE 6L

Protein	Organism	EC#	GenBank / GenPept		SwissProt	PDB / 3D
$\alpha$ -2,8 polysialyltransferase SiaD	<i>Neisseria meningitidis</i> B1940	2.4.-.-	M95053 X78068	AAA20478.1 CAA54985.1	Q51281 Q51145	
SynE	<i>Neisseria meningitidis</i> FAM18	n.d.	U75650	AAB53842.1	O06435	
polysialyltransferase (SiaD)(fragment)	<i>Neisseria meningitidis</i> M1019	n.d.	AY234192	AAO85290.1		
SiaD (fragment)	<i>Neisseria meningitidis</i> M209	n.d.	AY281046	AAP34769.1		
SiaD (fragment)	<i>Neisseria meningitidis</i> M3045	n.d.	AY281044	AAP34767.1		
polysialyltransferase (SiaD)(fragment)	<i>Neisseria meningitidis</i> M3315	n.d.	AY234191	AAO85289.1		
SiaD (fragment)	<i>Neisseria meningitidis</i> M3515	n.d.	AY281047	AAP34770.1		
polysialyltransferase (SiaD)(fragment)	<i>Neisseria meningitidis</i> M4211	n.d.	AY234190	AAO85288.1		
SiaD (fragment)	<i>Neisseria meningitidis</i> M4642	n.d.	AY281048	AAP34771.1		
polysialyltransferase (SiaD)(fragment)	<i>Neisseria meningitidis</i> M5177	n.d.	AY234193	AAO85291.1		
SiaD	<i>Neisseria meningitidis</i> M5178	n.d.	AY281043	AAP34766.1		
SiaD (fragment)	<i>Neisseria meningitidis</i> M980	n.d.	AY281045	AAP34768.1		
NMB0067	<i>Neisseria meningitidis</i> MC58	n.d.	NC_003112	NP_273131		
Lst	<i>Aeromonas punctata</i> Sch3	n.d.	AF126256	AAS66624.1		
ORF2	<i>Haemophilus influenzae</i> A2	n.d.	M94855	AAA24979.1		
HI1699	<i>Haemophilus influenzae</i> Rd	n.d.	U32842 NC_000907	AAC23345.1 NP_439841.1	Q48211	
$\alpha$ -2,3-sialyltransferase	<i>Neisseria gonorrhoeae</i> F62	2.4.99.4	U60664	AAC44539.1 AAE67205.1	P72074	
$\alpha$ -2,3-sialyltransferase	<i>Neisseria meningitidis</i> 126E, NRCC 4010	2.4.99.4	U60662	AAC44544.2		
$\alpha$ -2,3-sialyltransferase	<i>Neisseria meningitidis</i> 406Y, NRCC 4030	2.4.99.4	U60661	AAC44543.1		
$\alpha$ -2,3-sialyltransferase (NMB0922)	<i>Neisseria meningitidis</i> MC58	2.4.99.4	U60660 AE002443 NC_003112	AAC44541.1 AAF41330.1 NP_273962.1	P72097	
NMA1118	<i>Neisseria meningitidis</i> Z2491	n.d.	AL162755 NC_003116	CAB84380.1 NP_283887.1	Q9JUV5	
PM0508	<i>Pasteurella multocida</i> PM70	n.d.	AE006086 NC_002663	AAK02592.1 NP_245445.1	Q9CNC4	
WaaH	<i>Salmonella enterica</i> SARB25	n.d.	AF519787	AAM82550.1	Q8KS93	
WaaH	<i>Salmonella enterica</i> SARB3	n.d.	AF519788	AAM82551.1	Q8KS92	
WaaH	<i>Salmonella enterica</i> SARB39	n.d.	AF519789	AAM82552.1		
WaaH	<i>Salmonella enterica</i> SARB53	n.d.	AF519790	AAM82553.1		
WaaH	<i>Salmonella enterica</i> SARB57	n.d.	AF519791	AAM82554.1	Q8KS91	
WaaH	<i>Salmonella enterica</i> SARB71	n.d.	AF519793	AAM82556.1	Q8KS89	
WaaH	<i>Salmonella enterica</i>	n.d.	AF519792	AAM82555.1	Q8KS90	

FIGURE 6M

Protein	Organism	EC#	GenBank / GenPept	SwissProt	PDB / 3D
	SARB8				
WaaH	<i>Salmonella enterica</i> SARC10V	n.d.	AF519779	AAM88840.1	Q8KS99
WaaH (fragment)	<i>Salmonella enterica</i> SARC12	n.d.	AF519781	AAM88842.1	
WaaH (fragment)	<i>Salmonella enterica</i> SARC13I	n.d.	AF519782	AAM88843.1	Q8KS98
WaaH (fragment)	<i>Salmonella enterica</i> SARC14I	n.d.	AF519783	AAM88844.1	Q8KS97
WaaH	<i>Salmonella enterica</i> SARC15II	n.d.	AF519784	AAM88845.1	Q8KS96
WaaH	<i>Salmonella enterica</i> SARC16II	n.d.	AF519785	AAM88846.1	Q8KS95
WaaH (fragment)	<i>Salmonella enterica</i> SARC3I	n.d.	AF519772	AAM88834.1	Q8KSA4
WaaH (fragment)	<i>Salmonella enterica</i> SARC4I	n.d.	AF519773	AAM88835.1	Q8KSA3
WaaH	<i>Salmonella enterica</i> SARC5IIa	n.d.	AF519774	AAM88836.1	
WaaH	<i>Salmonella enterica</i> SARC6IIa	n.d.	AF519775	AAM88837.1	Q8KSA2
WaaH	<i>Salmonella enterica</i> SARC8	n.d.	AF519777	AAM88838.1	Q8KSA1
WaaH	<i>Salmonella enterica</i> SARC9V	n.d.	AF519778	AAM88839.1	Q8KSA0
UDP-glucose : $\alpha$ -1,2-glucosyltransferase (WaaH)	<i>Salmonella enterica</i> subsp. <i>arizonae</i> SARC 5	2.4.1.-	AF511116	AAM48166.1	
bifunctional $\alpha$ -2,3/-2,8-sialyltransferase (Cst-II)	<i>Campylobacter jejuni</i> ATCC 43449	n.d.	AF401529	AAL06004.1	Q93CZ5
Cst	<i>Campylobacter jejuni</i> 81-176	n.d.	AF305571	AAL09368.1	
$\alpha$ -2,3-sialyltransferase (Cst-III)	<i>Campylobacter jejuni</i> ATCC 43429	2.4.99.-	AY044156	AAK73183.1	
$\alpha$ -2,3-sialyltransferase (Cst-III)	<i>Campylobacter jejuni</i> ATCC 43430	2.4.99.-	AF400047	AAK85419.1	
$\alpha$ -2,3-sialyltransferase (Cst-II)	<i>Campylobacter jejuni</i> ATCC 43432	2.4.99.-	AF215659	AAG43979.1	Q9F0M9
$\alpha$ -2,3/8-sialyltransferase (CstII)	<i>Campylobacter jejuni</i> ATCC 43438	n.d.	AF400048	AAK91725.1	Q93MQ0
$\alpha$ -2,3-sialyltransferase cst-II	<i>Campylobacter jejuni</i> ATCC 43446	2.4.99.-	AF167344	AAF34137.1	
$\alpha$ -2,3-sialyltransferase (Cst-II)	<i>Campylobacter jejuni</i> ATCC 43456	2.4.99.-	AF401528	AAL05990.1	Q93D05
$\alpha$ -2,3/- $\alpha$ -2,8-sialyltransferase (CstII)	<i>Campylobacter jejuni</i> ATCC 43460	2.4.99.-	AY044868	AAK96001.1	Q938X6
$\alpha$ -2,3/8-sialyltransferase (Cst-II)	<i>Campylobacter jejuni</i> ATCC 700297	n.d.	AF216647	AAL36462.1	
ORF	<i>Campylobacter jejuni</i> GB11	n.d.	AY422197	AAR82875.1	
$\alpha$ -2,3-sialyltransferase cstIII	<i>Campylobacter jejuni</i> MSC57360	2.4.99.-	AF195055	AAG29922.1	
$\alpha$ -2,3-sialyltransferase cstIII Cj1140	<i>Campylobacter jejuni</i> NCTC 11168	2.4.99.-	AL139077 NC_002163	CAB73395.1 NP_282288.1	Q9PNF4
$\alpha$ -2,3/ $\alpha$ -2,8-sialyltransferase II (cstII)	<i>Campylobacter jejuni</i> O:10	n.d.	- AX934427	AAO96669.1 CAF04167.1	
$\alpha$ -2,3/ $\alpha$ -2,8-sialyltransferase II (CstII)	<i>Campylobacter jejuni</i> O:19	n.d.	AX934431	CAF04169.1	
$\alpha$ -2,3/ $\alpha$ -2,8-sialyltransferase II (CstII)	<i>Campylobacter jejuni</i> O:36	n.d.	AX934436	CAF04171.1	
$\alpha$ -2,3/ $\alpha$ -2,8-	<i>Campylobacter</i>	n.d.	AX934434	CAF04170.1	

FIGURE 6N

Protein	Organism	EC#	GenBank / GenPept		SwissProt	PDB / 3D
sialyltransferase II (CstII)	<i>jejuni</i> O:4					
$\alpha$ -2,3/ $\alpha$ -2,8-sialyltransferase II (CstII)	<i>Campylobacter jejuni</i> O:41	n.d.	-	AAO96670.1 AAT17967.1 AX934429 CAF04168.1		
$\alpha$ -2,3-sialyltransferase cst-I	<i>Campylobacter jejuni</i> OH4384	2.4.99.-	AF130466	AAF13495.1 AAS36261.1	Q9RGF1	
bifunctional $\alpha$ -2,3/-2,8-sialyltransferase (Cst-II)	<i>Campylobacter jejuni</i> OH4384	2.4.99.-	AF130984 AX934425	AAF31771.1 CAF04166.1	1RO7 1RO8	C A
HI0352 (fragment)	<i>Haemophilus influenzae</i> Rd	n.d.	U32720 X57315 NC_000907	AAC22013.1 CAA40567.1 NP_438516.1	P24324	
PM1174	<i>Pasteurella multocida</i> PM70	n.d.	AE006157 NC_002663	AAK03258.1 NP_246111.1	Q9CLP3	
Sequence 10 from patent US 6503744	Unknown.	n.d.	-	AAO96672.1		
Sequence 10 from patent US 6699705	Unknown.	n.d.	-	AAT17969.1		
Sequence 12 from patent US 6699705	Unknown.	n.d.	-	AAT17970.1		
Sequence 2 from patent US 6709834	Unknown.	n.d.	-	AAT23232.1		
Sequence 3 from patent US 6503744	Unknown.	n.d.	-	AAO96668.1		
Sequence 3 from patent US 6699705	Unknown.	n.d.	-	AAT17965.1		
Sequence 34 from patent US 6503744	Unknown.	n.d.	-	AAO96684.1		
Sequence 35 from patent US 6503744 (fragment)	Unknown.	n.d.	-	AAO96685.1 AAS36262.1		
Sequence 48 from patent US 6699705	Unknown.	n.d.	-	AAT17988.1		
Sequence 5 from patent US 6699705	Unknown.	n.d.	-	AAT17966.1		
Sequence 9 from patent US 6503744	Unknown.	n.d.	-	AAO96671.1		

**FIGURE 7A**

12AP1/E5 -- Viventia Biotech	AIDS vaccine -- ANRS, CIBG, Hesed
1964 -- Aventis	Biomed, Hollis-Eden, Rome, United
20K growth hormone -- AMUR	Biomedical, American Home Products,
28P6/E6 -- Viventia Biotech	Maxygen
3-Hydroxyphthaloyl-beta-lactoglobulin --	airway receptor ligand -- IC Innovations
4-IBB ligand gene therapy --	AJvW 2 -- Ajinomoto
64-Cu MAb conjugate TETA-1A3 --	AK 30 NGF -- Alkermes
Mallinckrodt Institute of Radiology	Albuferon -- Human Genome Sciences
64-Cu MAb conjugate TETA-cT84.66	albumin -- Biogen, DSM Anti-Infectives,
64-Cu Trastuzumab TETA conjugate --	Genzyme Transgenics, PPL Therapeutics,
Genentech	TranXenoGen, Welfide Corp.
A 200 -- Amgen	aldesleukin -- Chiron
A10255 -- Eli Lilly	alefacept -- Biogen
A1PDX -- Hedral Therapeutics	Alemtuzumab
A6 -- Angstrom	Allergy therapy -- ALK-Abello/Maxygen,
aaAT-III -- Genzyme	ALK-Abello/RP Scherer
Abciximab -- Centocor	allergy vaccines -- Allergy Therapeutics
ABI.001 -- Atlantic BioPharmaceuticals	Alnidofibatide -- Aventis Pasteur
ABT-828 -- Abbott	Alnorine -- SRC VB VECTOR
Accutin	ALP 242 -- Gruenenthal
Actinohivin	Alpha antitrypsin -- Arriva/Hyland
activin -- Biotech Australia, Human	Immuno/ProMetic/Protease Sciences
Therapeutics, Curis	Alpha-1 antitrypsin -- Cutter, Bayer, PPL
AD 439 -- Tanox	Therapeutics, Profile, ZymoGenetics,
AD 519 -- Tanox	Arriva
Adalimumab -- Cambridge Antibody Tech.	Alpha-1 protease inhibitor -- Genzyme
Adenocarcinoma vaccine -- Biomira -- NIS	Transgenics, Welfide Corp.
Adenosine deaminase -- Enzond	Alpha-galactose fusion protein --
Adenosine A2B receptor antagonists --	Immunomedics
Adenosine Therapeutics	Alpha-galactosidase A -- Research
ADP-001 -- Axis Genetics	Corporation Technologies, Genzyme
AF 13948 -- Affymax	Alpha-glucosidase -- Genzyme, Novazyme
Afelimomab -- Knoll	Alpha-lactalbumin
AFP-SCAN -- Immunomedics	Alpha-L-iduronidase -- Transkaryotic
AG 2195 -- Corixa	Therapies, BioMarin
agalsidase alfa -- Transkaryotic Therapies	alteplase -- Genentech
agalsidase beta -- Genzyme	alvircept sudotox -- NIH
AGENT-- Antisoma	ALX1-11 --sNPS Pharmaceuticals
AI 300 -- AutoImmune	Alzheimer's disease gene therapy
AI-101 -- Teva	AM-133 -- AMRAD
AI-102 -- Teva	Amb a 1 immunostim conj. -- Dynavax
AI-201 -- AutoImmune	AMD 3100 -- AnorMED -- NIS
AI-301 -- AutoImmune	AMD 3465 -- AnorMED -- NIS

**FIGURE 7B**

AMD 3465 – AnorMED -- NIS	Anti-B7-2 MAb GL-1
AMD Fab -- Genentech	Anti-B7-2-gelonin immunotoxin --
Amediplase -- Menarini, Novartis	Antibacterials/antifungals --
AM-F9	Diversa/IntraBiotics
Amoebiasis vaccine	Anti-beta-amyloid monoclonal antibodies --
Amphiregulin -- Octagene	Cambridge Antibody Tech., Wyeth-Ayerst
anakinra -- Amgen	Anti-BLyS antibodies -- Cambridge
analgesic -- Nobex	Antibody Tech. /Human Genome Sciences
ancestim -- Amgen	Antibody-drug conjugates -- Seattle
AnergiX.RA -- Corixa, Organon	Genetics/Eos
Angiocidin -- InKine	Anti-C5 MAb BB5-1 -- Alexion
angiogenesis inhibitors -- ILEX	Anti-C5 MAb N19-8 -- Alexion
AngioMab -- Antisoma	Anti-C8 MAb
Angiopoietins -- Regeneron/Procter &	anticancer cytokines -- BioPulse
Gamble	anticancer matrix -- Telios Integra
angiostatin -- EntreMed	Anticancer monoclonal antibodies -- ARIUS,
Angiostatin/endostatin gene therapy --	Immunex
Genetix Pharmaceuticals	anticancer peptides -- Maxygen, Micrologix
angiotensin-II, topical -- Maret	Anticancer prodrug Tech. -- Alexion
Anthrax -- EluSys Therapeutics/US Army	Antibody Technologies
Medical Research Institute	anticancer Troy-Bodies -- Affite -- Affitech
Anthrax vaccine	anticancer vaccine -- NIH
Anti platelet-derived growth factor D human	anticancers -- Epimmune
monoclonal antibodies -- CuraGen	Anti-CCR5/CXCR4 sheep MAb -- KS
Anti-17-1A MAb 3622W94 --	Biomedix Holdings
GlaxoSmithKline	Anti-CD11a MAb KBA --
Anti-2C4 MAb -- Genentech	Anti-CD11a MAb M17
anti-4-1BB monoclonal antibodies -- Bristol-	Anti-CD11a MAb TA-3 --
Myers Squibb	Anti-CD11a MAb WT.1 --
Anti-Adhesion Platform Tech. -- Cytovax	Anti-CD11b MAb -- Pharmacia
Anti-adipocyte MAb -- Cambridge Antibody	Anti-CD11b MAb LM2
Tech./ObeSys	Anti-CD154 MAb -- Biogen
antiallergics -- Maxygen	Anti-CD16-anti-CD30 MAb -- Biotest
antiallergy vaccine -- Acambis	Anti-CD18 MAb -- Pharmacia
Anti-alpha-4-integrin MAb	Anti-CD19 MAb B43 --
Anti-alphav $\beta$ 3 integrin MAb -- Applied	Anti-CD19 MAb -liposomal sodium butyrate
Molecular Evolution	conjugate --
Anti-angiogenesis monoclonal antibodies --	Anti-CD147
KS Biomedix/Schering AG	Anti-CD19 MAb-saporin conjugate --
Anti-B4 MAb-DC1 conjugate -- ImmunoGen	Anti-CD19-dsFv-PE38-immunotoxin --
Anti-B7 antibody PRIMATIZED -- IDEC	Anti-CD2 MAb 12-15 --
Anti-B7-1 MAb 16-10A1	Anti-CD2 MAb B-E2 -- Diaclone
Anti-B7-1 MAb 1G10	Anti-CD2 MAb OX34 --

**FIGURE 7C**

Anti-CD2 MAb OX54 –	Anti-CD4 MAb YTS 177-9
Anti-CD2 MAb OX55 –	Anti-CD40 ligand MAb 5c8 -- Biogen
Anti-CD2 MAb RM2-1	Anti-CD40 MAb
Anti-CD2 MAb RM2-2	Anti-CD40 MAb 5D12 – Tanox
Anti-CD2 MAb RM2-4	Anti-CD44 MAb A3D8
Anti-CD20 MAb BCA B20	Anti-CD44 MAb GKWA3
Anti-CD20-anti-Fc alpha RI bispecific MAb –	Anti-CD44 MAb IM7
Medarex, Tenovus	Anti-CD44 MAb KM81
Anti-CD22 MAb-saporin-6 complex –	Anti-CD44 variant monoclonal antibodies --
Anti-CD3 immunotoxin –	Corixa/Hebrew University
Anti-CD3 MAb 145-2C11 -- Pharming	Anti-CD45 MAb BC8-I-131
Anti-CD3 MAb CD4IgG conjugate --	Anti-CD45RB MAb
Genentech	Anti-CD48 MAb HuLy-m3
Anti-CD3 MAb humanised – Protein Design,	Anti-CD48 MAb WM-63
RW Johnson	Anti-CD5 MAb -- Becton Dickinson
Anti-CD3 MAb WT32	Anti-CD5 MAb OX19
Anti-CD3 MAb-ricin-chain-A conjugate –	Anti-CD6 MAb
Anti-CD3 MAb-xanthine-oxidase conjugate	Anti-CD7 MAb-PAP conjugate
–	Anti-CD7 MAb-ricin-chain-A conjugate
Anti-CD30 MAb BerH2 -- Medac	Anti-CD8 MAb – Amerimmune, Cytodyn,
Anti-CD30 MAb-saporin conjugate	Becton Dickinson
Anti-CD30-scFv-ETA'-immunotoxin	Anti-CD8 MAb 2-43
Anti-CD38 MAb AT13/5	Anti-CD8 MAb OX8
Anti-CD38 MAb-saporin conjugate	Anti-CD80 MAb P16C10 -- IDEC
Anti-CD3-anti-CD19 bispecific MAb	Anti-CD80 MAb P7C10 -- ID Vaccine
Anti-CD3-anti-EGFR MAb	Anti-CD8-idarubicin conjugate
Anti-CD3-anti-interleukin-2-receptor MAb	Anti-CEA MAb CE-25
Anti-CD3-anti-MOV18 MAb -- Centocor	Anti-CEA MAb MN 14 – Immunomedics
Anti-CD3-anti-SCLC bispecific MAb	Anti-CEA MAb MN14-PE40 conjugate –
Anti-CD4 idiotype vaccine	Immunomedics
Anti-CD4 MAb – Centocor, IDEC	Anti-CEA MAb T84.66-interleukin-2
Pharmaceuticals, Xenova Group	conjugate
Anti-CD4 MAb 16H5	Anti-CEA sheep MAb -- KS Biomedix
Anti-CD4 MAb 4162W94 -- GlaxoSmithKline	Holdings
Anti-CD4 MAb B-F5 -- Diaclone	Anti-cell surface monoclonal antibodies --
Anti-CD4 MAb GK1-5	Cambridge Antibody Tech. /Pharmacia
Anti-CD4 MAb KT6	Anti-c-erbB2-anti-CD3 bifunctional MAb --
Anti-CD4 MAb OX38	Otsuka
Anti-CD4 MAb PAP conjugate -- Bristol-	Anti-CMV MAb -- Scotgen
Myers Squibb	Anti-complement
Anti-CD4 MAb RIB 5-2	Anti-CTLA-4 MAb
Anti-CD4 MAb W3/25	Anti-EGFR catalytic antibody -- Hesed
Anti-CD4 MAb YTA 3.1.2	Biomed

**FIGURE 7D**

anti-EGFR immunotoxin -- IVAX	Anti-ICAM-1 MAb HA58
Anti-EGFR MAb -- Abgenix	Anti-ICAM-1 MAb YN1/1.7.4
Anti-EGFR MAb 528	Anti-ICAM-3 MAb ICM3 -- ICOS
Anti-EGFR MAb KSB 107 -- KS Biomedix	Anti-idiotypic breast cancer vaccine 11D10
Anti-EGFR MAb-DM1 conjugate -- ImmunoGen	Anti-idiotypic breast cancer vaccine ACA14C5 --
Anti-EGFR MAb-LA1 --	Anti-idiotypic cancer vaccine -- ImClone Systems/Merck KGaA ImClone, Viventia Biotech
Anti-EGFR sheep MAb -- KS Biomedix	Anti-idiotypic cancer vaccine 1A7 -- Titan
Anti-FAP MAb F19-I-131	Anti-idiotypic cancer vaccine 3H1 -- Titan
Anti-Fas IgM MAb CH11	Anti-idiotypic cancer vaccine TriAb -- Titan
Anti-Fas MAb Jo2	Anti-idiotypic Chlamydia trachomatis vaccine
Anti-Fas MAb RK-8	Anti-idiotypic colorectal cancer vaccine -- Novartis
Anti-Flt-1 monoclonal antibodies -- ImClone	Anti-idiotypic colorectal cancer vaccine -- Onyvax
Anti-fungal peptides -- State University of New York	Anti-idiotypic melanoma vaccine -- IDEC Pharmaceuticals
antifungal tripeptides -- BTG	Anti-idiotypic ovarian cancer vaccine ACA 125
Anti-ganglioside GD2 antibody-interleukin-2 fusion protein -- Lexigen	Anti-idiotypic ovarian cancer vaccine AR54 - AltaRex
Anti-GM2 MAb -- Kyowa	Anti-idiotypic ovarian cancer vaccine CA-125 -- AltaRex, Biomira
Anti-GM-CSF receptor monoclonal antibodies -- AMRAD	Anti-IgE catalytic antibody -- Hesed Biomed
Anti-gp130 MAb -- Tosoh	Anti-IgE MAb E26 -- Genentech
Anti-HCA monoclonal antibodies -- AltaRex/Epigen	Anti-IGF-1 MAb
Anti-hCG antibodies -- Abgenix/AVI BioPharma	anti-inflammatory -- GeneMax
Anti-heparanase human monoclonal antibodies -- Oxford Glycosciences/Medarex	anti-inflammatory peptide -- BTG
Anti-hepatitis C virus human monoclonal antibodies -- XTL Biopharmaceuticals	anti-integrin peptides -- Burnha
Anti-HER-2 antibody gene therapy	Anti-interferon-alpha-receptor MAb 64G12 -- Pharma Pacific Management
Anti-herpes antibody -- Epicyte	Anti-interferon-gamma MAb -- Protein Design Labs
Anti-HIV antibody -- Epicyte	Anti-interferon-gamma polyclonal antibody - Advanced Biotherapy
anti-HIV catalytic antibody -- Hesed Biomed	Anti-interleukin-10 MAb --
anti-HIV fusion protein -- Idun	Anti-interleukin-12 MAb --
anti-HIV proteins -- Cangene	Anti-interleukin-1-beta polyclonal antibody -- R&D Systems
Anti-HM1-24 MAb -- Chugai	Anti-interleukin-2 receptor MAb 2A3
Anti-hR3 MAb	
Anti-Human-Carcinoma-Antigen MAb -- Epicyte	
Anti-ICAM-1 MAb -- Boehringer Ingelheim	
Anti-ICAM-1 MAb 1A-29 -- Pharmacia	



**FIGURE 7E**

Anti-interleukin-2 receptor MAb 33B3-1 -- Immunotech	Anti-properdin monoclonal antibodies -- Abgenix/Gliatech
Anti-interleukin-2 receptor MAb ART-18	Anti-PSMA (prostate specific membrane antigen)
Anti-interleukin-2 receptor MAb LO-Tact-1	Anti-PSMA MAb J591 -- BZL Biologics
Anti-interleukin-2 receptor MAb Mikbeta1	Anti-Rev MAb gene therapy --
Anti-interleukin-2 receptor MAb NDS61	Anti-RSV antibodies -- Epicyte, Intracell
Anti-interleukin-4 MAb 11B11	Anti-RSV monoclonal antibodies -- Medarex/MedImmune, Applied Molecular Evolution/MedImmune
Anti-interleukin-5 MAb -- Wallace Laboratories	Anti-RSV MAb, inhalation -- Alkermes/MedImmune
Anti-interleukin-6 MAb -- Centocor, Diaclone, Pharmadigm	Anti-RT gene therapy
Anti-interleukin-8 MAb -- Abgenix	Antisense K-ras RNA gene therapy
Anti-interleukin-8 MAb -- Xenotech	Anti-SF-25 MAb
Anti-JL1 MAb	Anti-sperm antibody -- Epicyte
Anti-Klebsiella sheep MAb -- KS Biomedix Holdings	Anti-Tac(Fv)-PE38 conjugate
Anti-Laminin receptor MAb-liposomal doxorubicin conjugate	Anti-TAPA/CD81 MAb AMP1
Anti-LCG MAb -- Cytoclonal	Anti-tat gene therapy
Anti-lipopolysaccharide MAb -- VitaResc	Anti-TCR-alphabeta MAb H57-597
Anti-L-selectin monoclonal antibodies -- Protein Design Labs, Abgenix, Stanford University	Anti-TCR-alphabeta MAb R73
Anti-MBL monoclonal antibodies -- Alexion/Brigham and Women's Hospital	Anti-tenascin MAb BC-4-I-131
Anti-MHC monoclonal antibodies	Anti-TGF-beta human monoclonal antibodies -- Cambridge Antibody Tech., Genzyme
Anti-MIF antibody humanised -- IDEC, Cytokine PharmaSciences	Anti-TGF-beta MAb 2G7 -- Genentech
Anti-MRSA/VRSA sheep MAb -- KS Biomedix Holdings	Antithrombin III -- Genzyme Transgenics, Aventis, Bayer, Behringwerke, CSL, Myriad
Anti-mu MAb -- Novartis	Anti-Thy1 MAb
Anti-MUC-1 MAb	Anti-Thy1.1 MAb
Anti-MUC 18	Anti-tissue factor/factor VIIA sheep MAb -- KS Biomedix
Anti-Nogo-A MAb IN1	Anti-TNF monoclonal antibodies -- Centocor, Chiron, Peptech, Pharacia, Serono
Anti-nuclear autoantibodies -- Procyon	Anti-TNF sheep MAb -- KS Biomedix Holdings
Anti-ovarian cancer monoclonal antibodies - Dompe	Anti-TNFalpha MAb -- Genzyme
Anti-p185 monoclonal antibodies	Anti-TNFalpha MAb B-C7 -- Diaclone
Anti-p43 MAb	Anti-tooth decay MAb -- Planet BioTech.
Antiparasitic vaccines	Anti-TRAIL receptor-1 MAb -- Takeda
Anti-PDGF/bFGF sheep MAb -- KS Biomedix	Antitumour RNases -- NIH

**FIGURE 7F**

Anti-VCAM MAb 2A2 -- Alexion	Aurintricarboxylic acid-high molecular weight
Anti-VCAM MAb 3F4 -- Alexion	Autoimmune disorders -- GPC
Anti-VCAM-1 MAb	Biotech/MorphoSys
Anti-VEC MAb -- ImClone	Autoimmune disorders and transplant rejection -- Bristol-Myers Squibb/Genzyme
Anti-VEGF MAb -- Genentech	Tra
Anti-VEGF MAb 2C3	Autoimmune disorders/cancer --
Anti-VEGF sheep MAb -- KS Biomedix Holdings	Abgenix/Chiron, CuraGen
Anti-VLA-4 MAb HP1/2 -- Biogen	Autotaxin
Anti-VLA-4 MAb PS/2	Avicidin -- NeoRx
Anti-VLA-4 MAb R1-2	axogenesis factor-1 -- Boston Life Sciences
Anti-VLA-4 MAb TA-2	Axokine -- Regeneron
Anti-VAP-1 human MAb	B cell lymphoma vaccine -- Biomira
Anti-VRE sheep MAb -- KS Biomedix Holdings	B7-1 gene therapy --
ANUP -- TranXenoGen	BABS proteins -- Chiron
ANUP-1 -- Pharis	BAM-002 -- Novelos Therapeutics
AOP-RANTES -- Senetek	Basiliximab (anti CD25 MAb) -- Novartis
Apan-CH -- Praecis Pharmaceuticals	Bay-16-9996 -- Bayer
APC-8024 -- Demegen	Bay-39-9437 -- Bayer
ApoA-1 -- Milano, Pharmacia	Bay-50-4798 -- Bayer
Apogen -- Alexion	BB-10153 -- British Biotech
apolipoprotein A1 -- Avanir	BBT-001 -- Bolder BioTech.
Apolipoprotein E -- Bio-Tech. General	BBT-002 -- Bolder BioTech.
Applaggin -- Biogen	BBT-003 -- Bolder BioTech.
aprotinin -- ProdiGene	BBT-004 -- Bolder BioTech.
APT-070C -- AdProTech	BBT-005 -- Bolder BioTech.
AR 177 -- Aronex Pharmaceuticals	BBT-006 -- Bolder BioTech.
AR 209 -- Aronex Pharmaceuticals, Antigenics	BBT-007 -- Bolder BioTech.
AR545C	BCH-2763 -- Shire
ARGENT gene delivery systems -- ARIAD	BCSF -- Millenium Biologix
Arresten	BDNF -- Regeneron -- Amgen
ART-123 -- Asahi Kasei	Becaplermin -- Johnson & Johnson, Chiron
arylsulfatase B -- BioMarin	Bectumomab -- Immunomedics
Arylsulfatase B, Recombinant human -- BioMarin	Beriplast -- Aventis
AS 1051 -- Ajinomoto	Beta-adrenergic receptor gene therapy -- University of Arkansas
ASI-BCL -- Intracell	bFGF -- Scios
Asparaginase - Merck	BI 51013 -- Behringwerke AG
ATL-101 -- Alizyme	BIBH 1 -- Boehringer Ingelheim
Atrial natriuretic peptide -- Pharis	BIM-23190 -- Beaufour-Ipsen
	birch pollen immunotherapy -- Pharmacia
	bispecific fusion proteins -- NIH

**FIGURE 7G**

Bispecific MAb 2B1 -- Chiron	calcitonin -- oral -- Nobex, Emisphere,
Bitistatin	Pharmaceutical Discovery
BIWA 4 -- Boehringer Ingelheim	Calcitonin gene-related peptide -- Asahi
blood substitute -- Northfield, Baxter Intl.	Kasei -- Unigene
BLP-25 -- Biomira	calcitonin, human -- Suntory
BLS-0597 -- Boston Life Sciences	calcitonin, nasal -- Novartis, Unigene
BLYS -- Human Genome Sciences	calcitonin, Panoderm -- Elan
BLYS radiolabelled -- Human Genome	calcitonin, Peptitrol -- Shire
Sciences	calcitonin, salmon -- Therapicon
BM 06021 -- Boehringer Mannheim	calin -- Biopharm
BM-202 -- BioMarin	Calphobindin I
BM-301 -- BioMarin	calphobindin I -- Kowa
BM-301 -- BioMarin	calreticulin -- NYU
BM-302 -- BioMarin	Campath-1G
BMP 2 -- Genetics Institute/Medtronic-	Campath-1M
Sofamor Danek, Genetics Institute/	cancer therapy -- Cangene
Collagenesis, Genetics	cancer vaccine -- Aixlie, Aventis Pasteur,
Institute/Yamanouch	Center of Molecular Immunology ,YM
BMP 2 gene therapy	BioSciences, Cytos, Genzyme,
BMP 52 -- Aventis Pasteur, Biopharm	Transgenics, Globelimmune, Igeneon,
BMP-2 -- Genetics Institute	ImClone, Virogenetics, InterCell, Iomai,
BMS 182248 -- Bristol-Myers Squibb	Jenner Biotherapies, Memorial Sloan-
BMS 202448 -- Bristol-Myers Squibb	Kettering Cancer Center, Sydney Kimmel
bone growth factors -- IsoTis	Cancer Center, Novavax, Protein
BPC-15 -- Pfizer	Sciences, Argonex, SIGA
brain natriuretic peptide --	Cancer vaccine ALVAC-CEA B7.1 --
Breast cancer -- Oxford	Aventis Pasteur/Therion Biologics
GlycoSciences/Medarex	Cancer vaccine CEA-TRICOM -- Aventis
Breast cancer vaccine -- Therion Biologics,	Pasteur/Therion Biologics
Oregon	Cancer vaccine gene therapy -- Cantab
BSSL -- PPL Therapeutics	Pharmaceuticals
BST-2001 -- BioStratum	Cancer vaccine HER-2/neu -- Corixa
BST-3002 -- BioStratum	Cancer vaccine THERATOPE -- Biomira
BTI 322 --	cancer vaccine, PolyMASC -- Valentis
butyrylcholinesterase -- Shire	Candida vaccine -- Corixa, Inhibitex
C 6822 -- COR Therapeutics	Canstatin -- ILEX
C1 esterase inhibitor -- Pharming	CAP-18 -- Panorama
C3d adjuvant -- AdProTech	Cardiovascular gene therapy -- Collateral
CAB-2.1 -- Millennium	Therapeutics
calcitonin -- Inhale Therapeutics Systems,	carperitide -- Suntory
Aventis, Genetronics, TranXenoGen,	Casocidin-1 -- Pharis
Unigene, Rhone Poulenc Rohrer	CAT 152 -- Cambridge Antibody Tech.
	CAT 192 -- Cambridge Antibody Tech.

**FIGURE 7H**

CAT 213 -- Cambridge Antibody Tech.	Chlamydia pneumoniae vaccine -- Antex
Catalase-- Enzon	Biologics
Cat-PAD -- Circassia	Chlamydia trachomatis vaccine -- Antex
CB 0006 -- Celltech	Biologics
CCK(27-32)-- Akzo Nobel	Chlamydia vaccine -- GlaxoSmithKline
CCR2-64I -- NIH	Cholera vaccine CVD 103-HgR -- Swiss
CD, Procept -- Paligent	Serum and Vaccine Institute Berne
CD154 gene therapy	Cholera vaccine CVD 112 -- Swiss Serum
CD39 -- Immunex	and Vaccine Institute Berne
CD39-L2 -- Hyseq	Cholera vaccine inactivated oral -- SBL
CD39-L4 -- Hyseq	Vaccin
CD4 fusion toxin -- Senetek	Chrysalin -- Chrysalis BioTech.
CD4 IgG -- Genentech	CI-782 -- Hitachi Kase
CD4 receptor antagonists --	Ciliary neurotrophic factor -- Fidia, Roche
Pharmacopeia/Progenics	CIM project -- Active Biotech
CD4 soluble -- Progenics	CL 329753 -- Wyeth-Ayerst
CD4, soluble -- Genzyme Transgenics	CL22, Cobra -- ML Laboratories
CD40 ligand -- Immunex	Clenoliximab -- IDEC
CD4-ricin chain A -- Genentech	Clostridium difficile antibodies -- Epicyte
CD59 gene therapy -- Alexion	clotting factors -- Octagene
CD8 TIL cell therapy -- Aventis Pasteur	CMB 401 -- Celltech
CD8, soluble -- Avidex	CNTF -- Sigma-Tau
CD95 ligand -- Roche	Cocaine abuse vaccine -- Cantab,
CDP 571 -- Celltech	ImmuLogic, Scripps
CDP 850 -- Celltech	coccidiomycosis vaccine -- Arizo
CDP-860 (PEG-PDGF MAb) -- Celltech	collagen -- Type I -- Pharming
CDP 870 -- Celltech	Collagen formation inhibitors -- FibroGen
CDS-1 -- Ernest Orlando	Collagen/hydroxyapatite/bone growth factor
Cedelizumab -- Ortho-McNeil	-- Aventis Pasteur, Biopharm, Orquest
Cetermin -- Insmmed	collagenase -- BioSpecifics
CETP vaccine -- Avant	Colorectal cancer vaccine -- Wistar Institute
Cetrorelix	Component B, Recombinant -- Serono
Cetuximab	Connective tissue growth factor inhibitors --
CGH 400 -- Novartis	FibroGen/Taisho
CGP 42934 -- Novartis	Contortrostatin
CGP 51901 -- Tanox	contraceptive vaccine -- Zonagen
CGRP -- Unigene	Contraceptive vaccine hCG
CGS 27913 -- Novartis	Contraceptive vaccine male reversible --
CGS 32359 -- Novartis	IMMUCON
Chagas disease vaccine -- Corixa	Contraceptive vaccine zona pellucida --
chemokines -- Immune Response	Zonagen
CHH 380 -- Novartis	Copper-64 labelled MAb TETA-1A3 -- NCI
chitinase -- Genzyme, ICOS	Coralyne

**FIGURE 7I**

Corsevin M	Daclizumab (anti-IL2R MAb) – Protein
C-peptide analogues -- Schwarz	Design Labs
CPI-1500 -- Consensus	DAMP <sup>^</sup> -- Incyte Genomics
CRF -- Neurobiological Tech.	Daniplestim -- Pharmacia
cRGDfV pentapeptide –	darbepoetin alfa -- Amgen
CRL 1095 -- CytRx	DBI-3019 -- Diabetogen
CRL 1336 -- CytRx	DCC -- Genzyme
CRL 1605 -- CytRx	DDF -- Hyseq
CS-560 -- Sankyo	decorin – Integra, Telios
CSF -- ZymoGenetics	defensins -- Large Scale Biology
CSF-G – Hangzhou, Dong-A, Hanmi	DEGR-VIIa
CSF-GM – Cangene, Hunan, LG Chem	Delimmunised antibody 3B6/22 AGEN
CSF-M -- Zarix	Deimmunised anti-cancer antibodies --
CT 1579 – Merck Frosst	Biovation/Viragen
CT 1786 – Merck Frosst	Dendroamide A
CT-112 <sup>^</sup> -- BTG	Dengue vaccine -- Bavarian Nordic, Merck
CTB-134L -- Xenova	denileukin diftitox -- Ligand
CTC-111 -- Kaketsuken	DES-1101 -- Desmos
CTGF -- FibroGen	desirudin -- Novartis
CTLA4-Ig -- Bristol-Myers Squibb	desmopressin -- Unigene
CTLA4-Ig gene therapy –	Desmoteplase – Merck, Schering AG
CTP-37 -- AVI BioPharma	Destabilase
C-type natriuretic peptide -- Suntory	Diabetes gene therapy – DeveloGen, Pfizer
CVS 995 – Corvas Intl.	Diabetes therapy -- Crucell
CX 397 – Nikko Kyodo	Diabetes type 1 vaccine -- Diamyd
CY 1747 -- Epimmune	Therapeutics
CY 1748 -- Epimmune	DiaCIM -- YM BioSciences
Cyanovirin-N	dialytic oligopeptides -- Research Corp
Cystic fibrosis therapy -- CBR/IVAX	Diamyd -- Diamyd Therapeutics
CYT 351	DiaPep227-- Pepgen
cytokine Traps -- Regeneron	DiavaX -- Corixa
cytokines – Enzon, Cytoclonal	Digoxin MAb -- Glaxo
Cytomegalovirus glycoprotein vaccine –	Diphtheria tetanus pertussis-hepatitis B
Chiron, Aquila Biopharmaceuticals,	vaccine -- GlaxoSmithKline
Aventis Pasteur, Virogenetics	DIR therapy -- Solis Therapeutics –
Cytomegalovirus vaccine live -- Aventis	DNase -- Genentech
Pasteur	Dornase alfa -- Genentech
Cytosine deaminase gene therapy --	Dornase alfa, inhalation -- Genentech
GlaxoSmithKline	Doxorubicin-anti-CEA MAb conjugate –
DA-3003 -- Dong-A	Immunomedics
DAB389interleukin-6 -- Senetek	DP-107 -- Trimeris
DAB389interleukin-7	drotrecogin alfa -- Eli Lilly
	DTctGMCSF

**FIGURE 7J**

DTP-polio vaccine -- Aventis Pasteur	enzyme linked antibody nutrient depletion
DU 257-KM231 antibody conjugate --	therapy -- KS Biomedix Holdings
Kyowa	Eosinophil-derived neutralizing agent --
dural graft matrix -- Integra	EP-51216 -- Asta Medica
Dutepilase -- Baxter Intl.	EP-51389 -- Asta Medica
DWP-401 -- Daewoong	EPH family ligands -- Regeneron
DWP-404 -- Daewoong	Epidermal growth factor -- Hitachi Kasei,
DWP-408 -- Daewoong	Johnson & Johnson
Dx 88 (Epi-KAL2) -- Dyax	Epidermal growth factor fusion toxin --
Dx 890 (elastin inhibitors) -- Dyax	Senetek
E coli O157 vaccine -- NIH	Epidermal growth factor-genistein --
E21-R -- BresaGen	EPI-HNE-4 -- Dyax
Eastern equine encephalitis virus vaccine --	EPI-KAL2 -- Dyax
Echicetin --	Epoetin-alfa -- Amgen, Dragon
Echinhibin 1 --	Pharmaceuticals, Nanjing Huaxin
Echistatin -- Merck	Epratuzumab -- Immunomedics
Echitamine --	Epstein-Barr virus vaccine --
Ecromeximab -- Kyowa Hakko	Aviron/SmithKline Beecham, Bioreserch
EC-SOD -- PPL Therapeutics	Eptacog alfa -- Novo Nordisk
Eculizumab (5G1.1) -- Alexion	Eptifibatide -- COR Therapeutics
EDF -- Ajinomoto	erb-38 --
EDN derivative -- NIH	Erlizumab -- Genentech
EDNA -- NIH	erythropoietin -- Alkermes, ProLease, Dong-
Edobacomab -- XOMA	A, Elanex, Genetics Institute, LG Chem,
Edrecolomab -- Centocor	Protein Sciences, Serono, Snow Brand,
EF 5077	SRC VB VECTOR, Transkaryotic
Efalizumab -- Genentech	Therapies
EGF fusion toxin -- Seragen, Ligand	Erythropoietin Beta -- Hoffman La Roche
EGF-P64k vaccine -- Center of Molecular	Erythropoietin/Epoetin alfa -- Chugai
Immunology	Escherichia coli vaccine -- North American
EL 246 -- LigoCyte	Vaccine, SBL Vaccin, Swiss Serum and
elastase inhibitor -- Synergen	Vaccine Institute Berne
elcatonin -- Therapicon	etanercept -- Immunex
EMD 72000 -- Merck KGaA	examorelin -- Mediolanum
Emdogain -- BIORA	Exendin 4 -- Amylin
emfilermin -- AMRAD	exonuclease VII
Emoctakin -- Novartis	F 105 -- Centocor
enamel matrix protein -- BIORA	F-992 -- Fornix
Endo III -- NYU	Factor IX -- Alpha Therapeutics, Welfide
endostatin -- EntreMed, Pharis	Corp., CSL, enetics Institute/AHP,
Enhancins -- Micrologix	Pharmacia, PPL Therapeutics
Enlimomab -- Isis Pharm.	Factor IX gene therapy -- Cell Genesys
Enoxaparin sodium -- Pharmuka	

**FIGURE 7K**

Factor VII -- Novo Nordisk, Bayer, Baxter Intl.	follitropin alfa -- Alkermes, ProLease, PowderJect, Serono, Akzo Nobel
Factor VIIa -- PPL Therapeutics, ZymoGenetics	Follitropin Beta -- Bayer, Organon FP 59
Factor VIII -- Bayer Genentech, Beaufour-Ipsen, CLB, Inex, Octagen, Pharmacia, Pharming	FSH -- Ferring
Factor VIII -- PEGylated -- Bayer	FSH + LH -- Ferring
Factor VIII fragments -- Pharmacia	F-spondin -- CeNeS
Factor VIII gene therapy -- Targeted Genetics	fusion protein delivery system -- UAB Research Foundation
Factor VIII sucrose formulation -- Bayer, Genentech	fusion toxins -- Boston Life Sciences
Factor VIII-2 -- Bayer	G 5598 -- Genentech
Factor VIII-3 -- Bayer	GA-II -- Transkaryotic Therapies
Factor Xa inhibitors -- Merck, Novo Nordisk, Mochida	Gamma-interferon analogues -- SRC VB VECTOR
Factor XIII -- ZymoGenetics	Ganirelix -- Roche
Factors VIII and IX gene therapy -- Genetics Institute/Targeted Genetics	gastric lipase -- Meristem
Famoxin -- Genset	Gavilimomab --
Fas (delta) TM protein -- LXR BioTech.	G-CSF -- Amgen, SRC VB VECTOR
Fas TR -- Human Genome Sciences	GDF-1 -- CeNeS
Felvizumab -- Scotgen	GDF-5 -- Biopharm
FFR-VIIa -- Novo Nordisk	GDNF (glial derived neurotrophic factor) -- Amgen
FG-001 -- F-Gene	gelsolin -- Biogen
FG-002 -- F-Gene	Gemtuzumab ozogamicin -- Celltech
FG-004 -- F-Gene	Gene-activated epoetin-alfa -- Aventis Pharma -- Transkaryotic Therapies
FG-005 -- F-Gene	Glanzmann thrombasthenia gene therapy --
FGF + fibrin -- Repair	Glatiramer acetate -- Yeda
Fibrimage -- Bio-Tech. General	glial growth factor 2 -- CeNeS
fibrin-binding peptides -- ISIS Innovation	GLP-1 -- Amylin, Suntory, TheraTech, Watson
fibrinogen -- PPL Therapeutics, Pharming	GLP-1 peptide analogues -- Zealand Pharmaceuticals
fibroblast growth factor -- Chiron, NYU, Ramot, ZymoGenetics	glucagon -- Eli Lilly, ZymoGenetics
fibrolase conjugate -- Schering AG	Glucagon-like peptide-1 7-36 amide -- Suntory
Filgrastim -- Amgen	Glucogen-like peptide -- Amylin
filgrastim -- PDA modified -- Xencor	Glucocerebrosidase -- Genzyme
FLT-3 ligand -- Immunex	glutamate decarboxylase -- Genzyme Transgenics
FN18 CRM9 --	Glycoprotein S3 -- Kureha
follistatin -- Biotech Australia, Human Therapeutics	GM-CSF -- Immunex
	GM-CSF tumour vaccine -- PowderJect

**FIGURE 7L**

GnRH immunotherapeutic -- Protherics	Hemolink -- Hemosol
Goserelin (LhRH antagonist) -- AstraZeneca	hepapoietin -- Snow Brand
gp75 antigen -- ImClone	heparanase -- InSight
gp96 -- Antigenics	heparinase I -- Ibex
GPI 0100 -- Galenica	heparinase III -- Ibex
GR 4991W93 -- GlaxoSmithKline	Hepatitis A vaccine -- American Biogenetic Sciences
Granulocyte colony-stimulating factor -- Dong-A	Hepatitis A vaccine inactivated
Granulocyte colony-stimulating factor conjugate	Hepatitis A vaccine Nothav -- Chiron
grass allergy therapy -- Dynavax	Hepatitis A-hepatitis B vaccine -- GlaxoSmithKline
GRF1-44 -- ICN	hepatitis B therapy -- Tripep
Growth Factor -- Chiron, Atrigel, Atrix, Innogenetics, ZymoGenetics, Novo	Hepatitis B vaccine -- Amgen, Chiron SpA, Meiji Milk, NIS, Prodeva, PowderJect, Rhein Biotech
growth factor peptides -- Biotherapeutics	Hepatitis B vaccine recombinant -- Evans Vaccines, Epitec Combiotech, Genentech, MedImmune, Merck Sharp & Dohme, Rhein Biotech, Shantha Biotechnics, Vector, Yeda
growth hormone -- LG Chem	Hepatitis B vaccine recombinant TGP 943 -- Takeda
growth hormone, Recombinant human -- Serono	Hepatitis C vaccine -- Bavarian Nordic, Chiron, Innogenetics Acambis,
GT 4086 -- Giatech	Hepatitis D vaccine -- Chiron Vaccines
GW 353430 -- GlaxoSmithKline	Hepatitis E vaccine recombinant -- Genelabs/GlaxoSmithKline, Novavax
GW-278884 -- GlaxoSmithKline	hepatocyte growth factor -- Panorama, Sosei
H 11 -- Viventia Biotech	hepatocyte growth factor kringle fragments - - EntreMed
H5N1 influenza A virus vaccine -- Protein Sciences	Her-2/Neu peptides -- Corixa
haemoglobin -- Biopure	Herpes simplex glycoprotein DNA vaccine -- Merck, Wyeth-Lederle Vaccines-Malvern, Genentech, GlaxoSmithKline, Chiron, Takeda
haemoglobin 3011, Recombinant -- Baxter Healthcare	Herpes simplex vaccine -- Cantab Pharmaceuticals, CEL-SCI, Henderson Morley
haemoglobin crosfumaril -- Baxter Intl.	Herpes simplex vaccine live -- ImClone Systems/Wyeth-Lederle, Aventis Pasteur
haemoglobin stabilized -- Ajinomoto	HGF derivatives -- Dompe
haemoglobin, recombinant -- Apex	hIAPP vaccine -- Crucell
HAF -- Immune Response	
Hantavirus vaccine	
HB 19	
HBNF -- Regeneron	
HCC-1 -- Pharis	
hCG -- Milkhaus	
hCG vaccine -- Zonagen	
HE-317 -- Hollis-Eden Pharmaceuticals	
Heat shock protein cancer and influenza vaccines -- StressGen	
Helicobacter pylori vaccine -- Acambis, AstraZeneca/CSL, Chiron, Provalis	
Helistat-G -- GalaGen	



**FIGURE 7M**

Hib-hepatitis B vaccine -- Aventis Pasteur	host-vector vaccines -- Henogen
HIC 1	HPM 1 -- Chugai
HIP-- Altachem	HPV vaccine -- MediGene
Hirudins -- Biopharma, Cangene, Dongkook,	HSA -- Meristem
Japan Energy Corporation, Pharmacia	HSF -- StressGen
Corporation, SIR International, Sanofi-	HSP carriers --Weizmann, Yeda, Peptor
Synthelabo, Sotragene, Rhein Biotech	HSPPC-70 -- Antigenics
HIV edible vaccine -- ProdiGene	HSPPC-96, pathogen-derived -- Antigenics
HIV gp120 vaccine -- Chiron, Ajinomoto,	HSV 863 -- Novartis
GlaxoSmithKline, ID Vaccine, Progenics,	HTLV-I DNA vaccine
VaxGen	HTLV-I vaccine
HIV gp120 vaccine gene therapy --	HTLV-II vaccine -- Access
HIV gp160 DNA vaccine -- PowderJect,	HU 901 -- Tanox
Aventis Pasteur, Oncogen, Hyland	Hu23F2G -- ICOS
Immuno, Protein Sciences	HuHMFG1
HIV gp41 vaccine -- Panacos	HumaLYM -- Intracell
HIV HGP-30W vaccine -- CEL-SCI	Human krebs statika -- Yamanouchi
HIV immune globulin -- Abbott, Chiron	human monoclonal antibodies --
HIV peptides -- American Home Products	Abgenix/Biogen, Abgenix/ Corixa,
HIV vaccine -- Applied bioTech., Axis	Abgenix/Immune, Abgenix/Lexicon,
Genetics, Biogen, Bristol-Myers Squibb,	Abgenix/ Pfizer, Athersys/Medarex,
Genentech, Korea Green Cross, NIS,	Biogen/MorphoSys, CAT/Searle,
Oncogen, Protein Sciences Corporation,	Centocor/Medarex, Corixa/Kirin Brewery,
Terumo, Tonen Corporation, Wyeth-	Corixa/Medarex, Eos BioTech./Medarex,
Ayerst, Wyeth-Lederle Vaccines-Malvern,	Eos/Xenerex, Exelixis/Protein Design
Advanced BioScience Laboratories,	Labs, ImmunoGen/ Raven, Medarex/
Bavarian Nordic, Bavarian Nordic/Statens	B.Twelve, MorphoSys/ImmunoGen, XTL
Serum Institute, GeneCure, Immune	Biopharmaceuticals/Dyax,
Response, Progenics, Therion Biologics,	Human monoclonal antibodies --
United Biomedical, Chiron	Medarex/Northwest Biotherapeutics,
HIV vaccine vCP1433 -- Aventis Pasteur	Medarex/Seattle Genetics
HIV vaccine vCP1452 -- Aventis Pasteur	human netrin-1 -- Exelixis
HIV vaccine vCP205 -- Aventis Pasteur	human papillomavirus antibodies -- Epicyte
HL-9 -- American BioScience	Human papillomavirus vaccine -- Biotech
HM-9239 -- Cytran	Australia, IDEC, StressGen
HML-103 -- Hemosol	Human papillomavirus vaccine MEDI 501 --
HML-104 -- Hemosol	MedImmune/GlaxoSmithKline
HML-105 -- Hemosol	Human papillomavirus vaccine MEDI
HML-109 -- Hemosol	503/MEDI 504 --
HML-110 -- Hemosol	MedImmune/GlaxoSmithKline
HML-121 -- Hemosol	Human papillomavirus vaccine TA-CIN --
hNLP -- Pharis	Cantab Pharmaceuticals
Hookworm vaccine	

## FIGURE 7N

Human papillomavirus vaccine TA-HPV -- Cantab Pharmaceuticals	IL-7-Dap 389 fusion toxin -- Ligand
Human papillomavirus vaccine TH-GW -- Cantab/GlaxoSmithKline	IM-862 -- Cytran
human polyclonal antibodies -- Biosite/Eos BioTech./ Medarex	IMC-1C11 -- ImClone
human type II anti factor VIII monoclonal antibodies -- ThromboGenics	imiglucerase -- Genzyme
humanised anti glycoprotein Ib murine monoclonal antibodies -- ThromboGenics	Immune globulin intravenous (human) -- Hoffman La Roche
HumaRAD -- Intracell	immune privilege factor -- Proneuron
HuMax EGFR -- Genmab	Immunocal -- Immunotec
HuMax-CD4 -- Medarex	Immunogene therapy -- Briana Bio-Tech
HuMax-IL15 -- Genmab	Immunoliposomal 5-fluorodeoxyuridine- dipalmitate --
HYB 190 -- Hybridon	immunosuppressant vaccine -- Aixlie
HYB 676 -- Hybridon	immunotoxin -- Antisoma, NIH
I-125 MAb A33 -- Celltech	ImmuRAIT-Re-188 -- Immunomedics
Ibritumomab tiuxetan -- IDEC	imreg-1 -- Imreg
IBT-9401 -- Ibex	infertility -- Johnson & Johnson, E-TRANS
IBT-9402 -- Ibex	Infliximab -- Centocor
IC 14 -- ICOS	Influenza virus vaccine -- Aventis Pasteur, Protein Sciences
Idarubicin anti-Ly-2.1 --	inhibin -- Biotech Australia, Human Therapeutics
IDEC 114 -- IDEC	Inhibitory G protein gene therapy
IDEC 131 -- IDEC	INKP-2001 -- InKine
IDEC 152 -- IDEC	Inolimomab -- Diaclone
IDM 1 -- IDM	insulin -- AutoImmune, Altea, Biobras, BioSante, Bio-Tech. General, Chong Kun Dang, Emisphere, Flamel, Provalis, Rhein Biotech, TranXenoGen
IDPS -- Hollis-Eden Pharmaceuticals	insulin (bovine) -- Novartis
iduronate-2-sulfatase -- Transkaryotic Therapies	insulin analogue -- Eli Lilly
IGF/IBP-2-13 -- Pharis	Insulin Aspart -- Novo Nordisk
IGN-101 -- Igeneon	insulin detemir -- Novo Nordisk
IK HIR02 -- Iketon	insulin glargine -- Aventis
IL-11 -- Genetics Institute/AHP	insulin inhaled -- Inhale Therapeutics Systems, Alkermes
IL-13-PE38 -- NeoPharm	insulin oral -- Inovax
IL-17 receptor -- Immunex	insulin, AeroDose -- AeroGen
IL-18BP -- Yeda	insulin, AERx -- Aradigm
IL-1Hy1 -- Hyseq	insulin, BEODAS -- Elan
IL-1 $\beta$ -- Celltech	insulin, Biphasix -- Helix
IL-1 $\beta$ adjuvant -- Celltech	insulin, buccal -- Generex
IL-2 -- Chiron	insulin, I2R -- Flemington
IL-2 + IL-12 -- Hoffman La-Roche	insulin, intranasal -- Bentley
IL-6/sIL-6R fusion -- Hadasit	
IL-6R derivative -- Tosoh	

## FIGURE 70

insulin, oral – Nobex, Unigene	Interferon Gamma -- Boehringer Ingelheim,
insulin, Orasome -- Endorex	Sheffield, Rentschler, Hayashibara
insulin, ProMaxx -- Epic	interferon receptor , Type I -- Serono
insulin, Quadrant -- Elan	interferon(Gamma1B) -- Genentech
insulin, recombinant -- Aventis	Interferon-alpha-2b + ribavirin – Biogen,
insulin, Spiros -- Elan	ICN
insulin, Transfersome -- IDEA	Interferon-alpha-2b gene therapy --
insulin, Zymo, recombinant -- Novo Nordisk	Schering-Plough
insulinotropin -- Scios	Interferon-con1 gene therapy –
Insulysin gene therapy –	interleukin-1 antagonists -- Dompe
integrin antagonists -- Merck	Interleukin-1 receptor antagonist -- Abbott
interferon (Alpha2) -- SRC VB VECTOR,	Bioresearch, Pharmacia
Viragen, Dong-A, Hoffman La-Roche,	Interleukin-1 receptor type I -- Immunex
Genentech	interleukin-1 receptor Type II -- Immunex
interferon – BioMedicines, Human Genome	Interleukin-1 trap -- Regeneron
Sciences	Interleukin-1-alpha -- Immunex/Roche
interferon (Alfa-n3)—Interferon Sciences	interleukin-2 -- SRC VB VECTOR,
Intl.	Ajinomoto, Biomira, Chiron
interferon (Alpha), Biphasix -- Helix	IL-2/ diphtheria toxin -- Ligand
interferon (Alpha)—Amgen, BioNative,	Interleukin-3 -- Cangene
Novartis, Genzyme Transgenics,	Interleukin-4 -- Immunology Ventures,
Hayashibara, Inhale Therapeutics	Sanofi Winthrop, Schering-Plough,
Systems, Medusa, Flamel, Dong-A,	Immunex/ Sanofi Winthrop, Bayer, Ono
GeneTrol, Nastech, Shantha,	interleukin-4 + TNF-Alpha -- NIH
Wassermann, LG Chem, Sumitomo,	interleukin-4 agonist -- Bayer
Aventis, Behring EGIS, Pepgen, Servier,	interleukin-4 fusion toxin -- Ligand
Rhein Biotech,	Interleukin-4 receptor – Immunex, Immun
interferon (Alpha2A)	Interleukin-6 – Ajinomoto, Cangene, Yeda,
interferon (Alpha2B) – Enzon, Schering-	Genetics Institute, Novartis
Plough, Biogen, IDEA	interleukin-6 fusion protein
interferon (Alpha-N1) -- GlaxoSmithKline	interleukin-6 fusion toxin – Ligand, Serono
interferon (beta) – Rentschler, GeneTrol,	interleukin-7 -- IC Innovations
Meristem, Rhein Biotech, Toray, Yeda,	interleukin-7 receptor -- Immunex
Daiichi, Mochida	interleukin-8 antagonists -- Kyowa
interferon (Beta1A) – Serono, Biogen	Hakko/Millennium/Pfizer
interferon (beta1A),inhale -- Biogen	interleukin-9 antagonists -- Genaera
interferon (β1b)-- Chiron	Interleukin-10 – DNAX, Schering-Plough
interferon (tau)-- Pepgen	Interleukin-10 gene therapy –
Interferon alfacon-1 -- Amgen	interleukin-12 -- Genetics Institute, Hoffman
Interferon alpha-2a vaccine	La-Roche
Interferon Beta 1b -- Schering/Chiron,	interleukin-13 -- Sanofi
InterMune	interleukin-13 antagonists -- AMRAD
	Interleukin-13-PE38QQR

## FIGURE 7P

interleukin-15 -- Immunex	laronidase -- BioMarin
interleukin-16 -- Research Corp	Lassa fever vaccine
interleukin-18 -- GlaxoSmithKline	LCAT -- NIH
Interleukin-18 binding protein -- Serono	LDP 01 -- Millennium
lor-P3 -- Center of Molecular Immunology	LDP 02 -- Millennium
IP-10 -- NIH	Lecithinized superoxide dismutase --
IPF -- Metabolex	Seikagaku
IR-501 -- Immune Response	LeIF adjuvant -- Corixa
ISIS 9125 -- Isis Pharmaceuticals	leishmaniasis vaccine -- Corixa
ISURF No. 1554 -- Millennium	lenercept -- Hoffman La-Roche
ISURF No. 1866 -- Iowa State Univer.	Lenograstim -- Aventis, Chugai
ITF-1697 -- Italfarmaco	lepirudin -- Aventis
IxC 162 -- Ixion	leptin -- Amgen, IC Innovations
J 695 -- Cambridge Antibody Tech.,	Leptin gene therapy -- Chiron Corporation
Genetics Inst., Knoll	leptin, 2nd-generation -- Amgen
Jagged + FGF -- Repair	leridistim -- Pharmacia
JKC-362 -- Phoenix Pharmaceuticals	leuprolide, ProMaxx -- Epic
JTP-2942 -- Japan Tobacce	leuprorelin, oral -- Unigene
Juman monoclonal antibodies --	LeuTech -- Papatin
Medarex/Raven	LEX 032 -- SuperGen
K02 -- Axys Pharmaceuticals	LiDEPT -- Novartis
Keliximab -- IDEC	Lintuzumab (anti-CD33 MAb) -- Protein
Keyhole limpet haemocyanin	Design Labs
KGF -- Amgen	lipase -- Altus Biologics
KM 871 -- Kyowa	lipid A vaccine -- Entremed
KPI 135 -- Scios	lipid-linked anchor Tech. -- ICRT, ID
KPI-022 -- Scios	Biomedical
Kringle 5	liposome-CD4 Tech. -- Sheffield
KSB 304	Listeria monocytogenes vaccine
KSB-201 -- KS Biomedix	LMB 1
L 696418 -- Merck	LMB 7
L 703801 -- Merck	LMB 9 -- Battelle Memorial Institute, NIH
L1 -- Acorda	LM-CD45 -- Cantab Pharmaceuticals
L-761191 -- Merck	lovastatin -- Merck
lactoferrin -- Meristem, Pharming, Agennix	LSA-3
lactoferrin cardio -- Pharming	LT- $\beta$ receptor -- Biogen
LAG-3 -- Serono	lung cancer vaccine -- Corixa
LAIT -- GEMMA	lusupultide -- Scios
LAK cell cytotoxin -- Arizona	L-Vax -- AVAX
lamellarins -- PharmaMar/University of	LY 355455 -- Eli Lilly
Malaga	LY 366405 -- Eli Lilly
laminin A peptides -- NIH	LY-355101 -- Eli Lilly
lanotepase -- Genetics Institute	

**FIGURE 7Q**

Lyme disease DNA vaccine -- Vical/Aventis Pasteur	MDX 240 -- Medarex
Lyme disease vaccine -- Aquila Biopharmaceuticals, Aventis, Pasteur, Symbicom, GlaxoSmithKline, Hyland Immuno, MedImmune	MDX 33
Lymphocytic choriomeningitis virus vaccine	MDX 44 -- Medarex
lymphoma vaccine -- Biomira, Genitope	MDX 447 -- Medarex
LYP18	MDX H210 -- Medarex
lys plasminogen, recombinant	MDX RA -- Houston BioTech., Medarex
Lysosomal storage disease gene therapy -- Avigen	ME-104 -- Pharmexa
lysostaphin -- Nutrition 21	Measles vaccine
M 23 -- Gruenenthal	Mecasermin -- Cephalon/Chiron, Chiron
M1 monoclonal antibodies -- Acorda Therapeutics	MEDI 488 -- MedImmune
MA 16N7C2 -- Corvas Intl.	MEDI 500
malaria vaccine -- GlaxoSmithKline, AdProTech, Antigenics, Apovia, Aventis Pasteur, Axis Genetics, Behringwerke, CDCP, Chiron Vaccines, Genzyme Transgenics, Hawaii, MedImmune, NIH, NYU, Oxxon, Roche/Saramane, Biotech Australia, Rx Tech	MEDI 507 -- BioTransplant
Malaria vaccine CDC/NIIMALVAC-1	melanin concentrating hormone -- Neurocrine Biosciences
malaria vaccine, multicomponent	melanocortins -- OMRF
mammaglobin -- Corixa	Melanoma monoclonal antibodies -- Viragen
mammastatin -- Biotherapeutics	melanoma vaccine -- GlaxoSmithKline, Akzo Nobel, Avant, Aventis Pasteur, Bavarian Nordic, Biovector, CancerVax, Genzyme Molecular Oncology, Humbolt, ImClone Systems, Memorial, NYU, Oxxon
mannan-binding lectin -- NatImmu	Melanoma vaccine Magevac -- Therion
mannan-MUC1 -- Psiron	memory enhancers -- Scios
MAP 30	meningococcal B vaccine -- Chiron
Marinovir -- Phytera	meningococcal vaccine -- CAMR
MARstem -- Maret	Meningococcal vaccine group B conjugate - North American Vaccine
MB-015 -- Mochida	Meningococcal vaccine group B recombinant -- BioChem Vaccines, Microscience
MBP -- ImmuLogic	Meningococcal vaccine group Y conjugate - North American Vaccine
MCI-028 -- Mitsubishi-Tokyo	Meningococcal vaccine groups A B and C conjugate -- North American Vaccine
MCIF -- Human Genome Sciences	Mepolizumab -- GlaxoSmithKline
MDC -- Advanced BioScience -- Akzo Nobel, ICOS	Metastatin -- EntreMed, Takeda
MDX 11 -- Medarex	Met-Ckb7 -- Human Genome Sciences
MDX 210 -- Medarex	met-enkephalin -- TNI
MDX 22 -- Medarex	METH-1 -- Human Genome Sciences
MDX 22	methioninase -- AntiCancer
	Methionine lyase gene therapy -- AntiCancer

## FIGURE 7R

Met-RANTES -- Genexa Biomedical, Serono	MAb 323A3 -- Centocor
Metreleptin	MAb 3C5
Microtubule inhibitor MAb	MAb 3F12
Immunogen/Abgenix	MAb 3F8
MGDF -- Kirin	MAb 42/6
MGV -- Progenics	MAb 425 -- Merck KGaA
micrin -- Endocrine	MAb 447-52D -- Merck Sharp & Dohme
microplasmin -- ThromboGenics	MAb 45-2D9- -- haematoporphyrin conjugate
MIF -- Genetics Institute	MAb 4B4
migration inhibitory factor -- NIH	MAb 4E3-CPA conjugate -- BCM Oncologia
Mim CD4.1 -- Xycte Therapies	MAb 4E3-daunorubicin conjugate
mirostipen -- Human Genome Sciences	MAb 50-6
Mitumomab (BEC-2) -- ImClone Systems, Merck KGaA	MAb 50-61A -- Institut Pasteur
MK 852 -- Merck	MAb 5A8 -- Biogen
MLN 1202 (Anti-CCR2 monoclonal antibody) -- Millenium Pharmaceuticals	MAb 791T/36-methotrexate conjugate
Mobenakin -- NIS	MAb 7c11.e8
molgramostim -- Genetics Institute, Novartis	MAb 7E11 C5-selenocystamine conjugate
monoclonal antibodies -- Abgenix/Celltech, Immusol/ Medarex, Viragen/ Roslin Institute, Cambridge Antibody Tech./Elan	MAb 93KA9 -- Novartis
MAb 108 --	MAb A5B7-cisplatin conjugate -- Biodynamics Research, Pharmacia
MAb 10D5 --	MAb A5B7-I-131
MAb 14.18-interleukin-2 immunocytokine -- Lexigen	MAb A7
MAb 14G2a --	MAb A717 -- Exocell
MAb 15A10 --	MAb A7-zinostatin conjugate
MAb 170 -- Biomira	MAb ABX-RB2 -- Abgenix
MAb 177Lu CC49 --	MAb ACA 11
MAb 17F9	MAb AFP-I-131 -- Immunomedics
MAb 1D7	MAb AP1
MAb 1F7 -- Immune Network	MAb AZ1
MAb 1H10-doxorubicin conjugate	MAb B3-LysPE40 conjugate
MAb 26-2F	MAb B4 -- United Biomedical
MAb 2A11	MAb B43 Genistein-conjugate
MAb 2E1 -- RW Johnson	MAb B43.13-Tc-99m -- Biomira
MAb 2F5	MAb B43-PAP conjugate
MAb 31.1 -- International BioImmune Systems	MAb B4G7-gelonin conjugate
MAb 32 -- Cambridge Antibody Tech., Peptech	MAb BCM 43-daunorubicin conjugate -- BCM Oncologia
	MAb BIS-1
	MAb BMS 181170 -- Bristol-Myers Squibb
	MAb BR55-2
	MAb BW494
	MAb C 242-DM1 conjugate -- ImmunoGen

**FIGURE 7S**

MAB C242-PE conjugate	MAB KS1-4-methotrexate conjugate
MAB c30-6	MAB L6 -- Bristol-Myers Squibb, Oncogen
MAB CA208-cytorhodin-S conjugate -- Hoechst Japan	MAB LiCO 16-88
MAB CC49 -- Enzon	MAB LL2-I-131 -- Immunomedics
MAB ch14.18 --	MAB LL2-Y-90
MAB CH14.18-GM-CSF fusion protein -- Lexigen	MAB LS2D617 -- Hybritech
MAB chCE7	MAB LYM-1-gelonin conjugate
MAB CI-137 -- AMRAD	MAB LYM-1-I-131
MAB cisplatin conjugate	MAB LYM-1-Y-90
MAB CLB-CD19	MAB LYM-2 -- Peregrine
MAB CLB-CD19v	MAB M195
MAB CLL-1 -- Peregrine	MAB M195-bismuth 213 conjugate -- Protein Design Labs
MAB CLL-1-GM-CSF conjugate	MAB M195-gelonin conjugate
MAB CLL-1-IL-2 conjugate -- Peregrine	MAB M195-I-131
MAB CLN IgG -- doxorubicin conjugates	MAB M195-Y-90
MAB conjugates -- Tanox	MAB MA 33H1 -- Sanofi
MAB D612	MAB MAD11
MAB Dal B02	MAB MGb2
MAB DC101 -- ImClone	MAB MINT5
MAB EA 1 --	MAB MK2-23
MAB EC708 -- Biovation	MAB MOC31 ETA(252-613) conjugate
MAB EP-5C7 -- Protein Design Labs	MAB MOC-31-In-111
MAB ERIC-1 -- ICRT	MAB MOC-31-PE conjugate
MAB F105 gene therapy	MAB MR6 --
MAB FC 2.15	MAB MRK-16 -- Aventis Pasteur
MAB G250 -- Centocor	MAB MS11G6
MAB GA6	MAB MX-DTPA BrE-3
MAB GA733	MAB MY9
MAB Gliomab-H -- Viventia Biotech	MAB Nd2 -- Tosoh
MAB HB2-saporin conjugate	MAB NG-1 -- Hygeia
MAB HD 37 --	MAB NM01 -- Nissin Food
MAB HD37-ricin chain-A conjugate	MAB OC 125
MAB HNK20 -- Acambis	MAB OC 125-CMA conjugate
MAB huN901-DM1 conjugate -- ImmunoGen	MAB OKI-1 -- Ortho-McNeil
MAB I-131 CC49 -- Corixa	MAB OX52 -- Bioproducts for Science
MAB ICO25	MAB PMA5
MAB ICR12-CPG2 conjugate	MAB PR1
MAB ICR-62	MAB prost 30
MAB IRac-ricin A conjugate	MAB R-24
MAB K1	MAB R-24 $\alpha$ Human GD3 -- Celltech
	MAB RFB4-ricin chain A conjugate
	MAB RFT5-ricin chain A conjugate

**FIGURE 7T**

MAb SC 1	mucosal tolerance -- Aberdeen
MAb SM-3 -- ICRT	mullerian inhibiting subst
MAb SMART 1D10 -- Protein Design Labs	muplestim -- Genetics Institute, Novartis,
MAb SMART ABL 364 -- Novartis	DSM Anti-Infectives
MAb SN6f	murine MAb -- KS Biomedix
MAb SN6f-deglycosylated ricin A chain	Mutant somatropin -- JCR Pharmaceutical
conjugate --	MV 833 -- Toagosei
MAb SN6j	Mycoplasma pulmonis vaccine
MAb SN7-ricin chain A conjugate	Mycoprex -- XOMA
MAb T101-Y-90 conjugate -- Hybritech	myeloperoxidase -- Henogen
MAb T-88 -- Chiron	myostatin -- Genetics Institute
MAb TB94 -- Cancer ImmunoBiology	Nacolomab tafenatox -- Pharmacia
MAb TEC 11	Nagrecor -- Scios
MAb TES-23 -- Chugai	nagrestipen -- British Biotech
MAb TM31 -- Avant	NAP-5 -- Corvas Intl.
MAb TNT-1 -- Cambridge Antibody Tech.,	NAPc2 -- Corvas Intl.
Peregrine	nartograstim -- Kyowa
MAb TNT-3	Natalizumab -- Protein Design Labs
MAb TNT-3 -- IL2 fusion protein --	Nateplase -- NIH, Nihon Schering
MAb TP3-At-211	nateplase -- Schering AG
MAb TP3-PAP conjugate --	NBI-3001 -- Neurocrine Biosci.
MAb UJ13A -- ICRT	NBI-5788 -- Neurocrine Biosci.
MAb UN3	NBI-6024 -- Neurocrine Biosci.
MAb ZME-018-gelonin conjugate	Nef inhibitors -- BRI
MAb-BC2 -- GlaxoSmithKline	Neisseria gonorrhoea vaccine -- Antex
MAb-DM1 conjugate -- ImmunoGen	Biologics
MAb-ricin-chain-A conjugate -- XOMA	Neomycin B-arginine conjugate
MAb-temoporfin conjugates	Nerelimomab -- Chiron
Monopharm C -- Viventia Biotech	Nerve growth factor -- Amgen -- Chiron,
monteplase -- Eisai	Genentech
montirelin hydrate -- Gruenenthal	Nerve growth factor gene therapy
moroctocog alfa -- Genetics Institute	nesiritide citrate -- Scios
Moroctocog-alfa -- Pharmacia	neuregulin-2 -- CeNeS
MP 4	neurocan -- NYU
MP-121 -- Biopharm	neuronal delivery system -- CAMR
MP-52 -- Biopharm	Neurophil inhibitory Factor -- Corvas
MRA -- Chugai	Neuroprotective vaccine -- University of
MS 28168 -- Mitsui Chemicals, Nihon	Auckland
Schering	neurotrophic chimaeras -- Regeneron
MSH fusion toxin -- Ligand	neurotrophic factor -- NsGene, CereMedix
MSI-99 -- Genaera	NeuroVax -- Immune Response
MT 201 -- Micromet	neurturin -- Genentech
Muc-1 vaccine -- Corixa	neutral endopeptidase -- Genentech



## FIGURE 7U

NGF enhancers -- NeuroSearch	onychomycosis vaccine -- Boehringer
NHL vaccine -- Large Scale Biology	Ingelheim
NIP45 -- Boston Life Sciences	opebecan -- XOMA
NKI-B20	opioids -- Arizona
NM 01 -- Nissin Food	Oprelvekin -- Genetics Institute
NMI-139 -- NitroMed	Oregovomab -- AltaRex
NMMP -- Genetics Institute	Org-33408 b-- Akzo Nobel
NN-2211 -- Novo Nordisk	Orolip DP -- EpiCept
Noggin -- Regeneron	oryzacystatin
Nonacog alfa	OSA peptides -- GenSci Regeneration
Norelin -- Biostar	osteoblast-cadherin GF -- Pharis
Norwalk virus vaccine	Osteocalcin-thymidine kinase gene therapy
NRLU 10 -- NeoRx	osteogenic protein -- Curis
NRLU 10 PE -- NeoRx	osteopontin -- OraPharma
NT-3 -- Regeneron	osteoporosis peptides -- Integra, Telios
NT-4/5 -- Genentech	osteoprotegerin -- Amgen, SnowBrand
NU 3056	otitis media vaccines -- Antex Biologics
NU 3076	ovarian cancer -- University of Alabama
NX 1838 -- Gilead Sciences	OX40-IgG fusion protein -- Cantab, Xenova
NY ESO-1/CAG-3 antigen -- NIH	P 246 -- Diatide
NYVAC-7 -- Aventis Pasteur	P 30 -- Alfacell
NZ-1002 -- Novazyme	p1025 -- Active Biotech
obesity therapy -- Nobex	P-113 <sup>^</sup> -- Demegen
OC 10426 -- Ontogen	P-16 peptide -- Transition Therapeutics
OC 144093 -- Ontogen	p43 -- Ramot
OCIF -- Sankyo	P-50 peptide -- Transition Therapeutics
Oct-43 -- Otsuka	p53 + RAS vaccine -- NIH, NCI
Odulimomab -- Immunotech	PACAP(1-27) analogue
OK PSA - liposomal	paediatric vaccines -- Chiron
OKT3-gamma-1-ala-ala	Pafase -- ICOS
OM 991	PAGE-4 plasmid DNA -- IDEC
OM 992	PAI-2 -- Biotech Australia, Human
Omalizumab -- Genentech	Therapeutics
oncoimmunin-L -- NIH	Palifermin (keratinocyte growth factor) --
Oncolysin B -- ImmunoGen	Amgen
Oncolysin CD6 -- ImmunoGen	Palivizumab -- MedImmune
Oncolysin M -- ImmunoGen	PAM 4 -- Merck
Oncolysin S -- ImmunoGen	pamiteplase -- Yamanouchi
Oncophage -- Antigenics	pancreatin, Minitabs -- Eurand
Oncostatin M -- Bristol-Myers Squibb	Pangen -- Fournier
OncoVax-CL -- Jenner Biotherapies	Pantarin -- Selective Genetics
OncoVax-P -- Jenner Biotherapies	Parainfluenza virus vaccine -- Pharmacia,
onercept -- Yeda	Pierre Fabre

**FIGURE 7V**

paraoxanase -- Esperion	peptide vaccine -- NIH ,NCI
parathyroid hormone -- Abiogen, Korea	Pexelizumab
Green Cross	pexiganan acetate -- Genaera
Parathyroid hormone (1-34) --	Pharmaprojects No. 3179 -- NYU
Chugai/Suntory	Pharmaprojects No. 3390 -- Ernest Orlando
Parkinson's disease gene therapy -- Cell	Pharmaprojects No. 3417 -- Sumitomo
Genesys/ Ceregene	Pharmaprojects No. 3777 -- Acambis
Parvovirus vaccine -- MedImmune	Pharmaprojects No. 4209 -- XOMA
PCP-Scan -- Immunomedics	Pharmaprojects No. 4349 -- Baxter Intl.
PDGF -- Chiron	Pharmaprojects No. 4651
PDGF cocktail -- Theratechnologies	Pharmaprojects No. 4915 -- Avanir
peanut allergy therapy -- Dynavax	Pharmaprojects No. 5156 -- Rhizogenics
PEG anti-ICAM MAb -- Boehringer	Pharmaprojects No. 5200 -- Pfizer
Ingelheim	Pharmaprojects No. 5215 -- Origene
PEG asparaginase -- Enzon	Pharmaprojects No. 5216 -- Origene
PEG glucocerebrosidase	Pharmaprojects No. 5218 -- Origene
PEG hirudin -- Knoll	Pharmaprojects No. 5267 -- ML
PEG interferon-alpha-2a -- Roche	Laboratories
PEG interferon-alpha-2b + ribavirin --	Pharmaprojects No. 5373 -- MorphoSys
Biogen, Enzon, ICN Pharmaceuticals,	Pharmaprojects No. 5493 -- Metabolex
Schering-Plough	Pharmaprojects No. 5707 -- Genentech
PEG MAb A5B7 --	Pharmaprojects No. 5728 -- Autogen
Pegacaristim -- Amgen -- Kirin Brewery --	Pharmaprojects No. 5733 -- BioMarin
ZymoGenetics	Pharmaprojects No. 5757 -- NIH
Pegaldesleukin -- Research Corp	Pharmaprojects No. 5765 -- Gryphon
pegaspargase -- Enzon	Pharmaprojects No. 5830 -- AntiCancer
pegfilgrastim -- Amgen	Pharmaprojects No. 5839 -- Dyax
PEG-interferon Alpha -- Viragen	Pharmaprojects No. 5849 -- Johnson &
PEG-interferon Alpha 2A -- Hoffman La-	Johnson
Roche	Pharmaprojects No. 5860 -- Mitsubishi-
PEG-interferon Alpha 2B -- Schering-	Tokyo
Plough	Pharmaprojects No. 5869 -- Oxford
PEG-r-hirudin -- Abbott	GlycoSciences
PEG-rHuMGDF -- Amgen	Pharmaprojects No. 5883 -- Asahi Brewery
PEG-uricase -- Mountain View	Pharmaprojects No. 5947 -- StressGen
Pegvisomant -- Genentech	Pharmaprojects No. 5961 --
PEGylated proteins, PolyMASC -- Valentis	Theratechnologies
PEGylated recombinant native human leptin	Pharmaprojects No. 5962 -- NIH
-- Roche	Pharmaprojects No. 5966 -- NIH
Pemtumomab	Pharmaprojects No. 5994 -- Pharming
Penetratin -- Cyclacel	Pharmaprojects No. 5995 -- Pharming
Pepscan -- Antisoma	Pharmaprojects No. 6023 -- IMMUCON
peptide G -- Peptech, ICRT	Pharmaprojects No. 6063 -- Cytoclinal

## FIGURE 7W

Pharmaprojects No. 6073 -- SIDDCO	Pneumococcal vaccine -- Antex Biologics,
Pharmaprojects No. 6115 -- Genzyme	Aventis Pasteur
Pharmaprojects No. 6227 -- NIH	Pneumococcal vaccine intranasal --
Pharmaprojects No. 6230 -- NIH	BioChem Vaccines/Biovector
Pharmaprojects No. 6236 -- NIH	PR1A3
Pharmaprojects No. 6243 -- NIH	PR-39
Pharmaprojects No. 6244 -- NIH	pralmorelin -- Kaken
Pharmaprojects No. 6281 -- Senetek	Pretarget-Lymphoma -- NeoRx
Pharmaprojects No. 6365 -- NIH	Priliximab -- Centocor
Pharmaprojects No. 6368 -- NIH	PRO 140 -- Progenics
Pharmaprojects No. 6373 -- NIH	PRO 2000 -- Procept
Pharmaprojects No. 6408 -- Pan Pacific	PRO 367 -- Progenics
Pharmaprojects No. 6410 -- Athersys	PRO 542 -- Progenics
Pharmaprojects No. 6421 -- Oxford	pro-Apo A-I -- Esperion
GlycoSciences	prolactin -- Genzyme
Pharmaprojects No. 6522 -- Maxygen	Prosaptide TX14(A) -- Bio-Tech. General
Pharmaprojects No. 6523 -- Pharis	prostate cancer antibodies -- Immunex,
Pharmaprojects No. 6538 -- Maxygen	UroCor
Pharmaprojects No. 6554 -- APALEXO	prostate cancer antibody therapy --
Pharmaprojects No. 6560 -- Ardana	Genentech/UroGenesys,
Pharmaprojects No. 6562 -- Bayer	Genotherapeutics
Pharmaprojects No. 6569 -- Eos	prostate cancer immunotherapeutics -- The
Phenoxazine	PSMA Development Company
Phenylase -- Ibex	prostate cancer vaccine -- Aventis Pasteur,
Pigment epithelium derived factor --	Zonagen, Corixa, Dendreon, Jenner
plasminogen activator inhibitor-1,	Biotherapies, Therion Biologics
recombinant -- DuPont Pharmaceuticals	prostate-specific antigen -- EntreMed
Plasminogen activators -- Abbott	protein A -- RepliGen
Laboratories, American Home Products,	protein adhesives -- Enzon
Boehringer Mannheim, Chiron	protein C -- Baxter Intl., PPL Therapeutics,
Corporation, DuPont Pharmaceuticals, Eli	ZymoGenetics
Lilly, Shionogi, Genentech, Genetics	protein C activator -- Gilead Sciences
Institute, GlaxoSmithKline, Hemispherx	protein kinase R antags -- NIH
Biopharma, Merck & Co, Novartis,	protirelin -- Takeda
Pharmacia Corporation, Wakamoto, Yeda	protocadherin 2 -- Caprion
plasminogen-related peptides -- Bio-Tech.	Pro-urokinase -- Abbott, Bristol-Myers
General/MGH	Squibb, Dainippon, Tosoh -- Welfide
platelet factor 4 -- RepliGen	P-selectin glycoprotein ligand-1 -- Genetics
Platelet-derived growth factor -- Amgen --	Institute
ZymoGenetics	pseudomonal infections -- InterMune
plusonermin-- Hayashibara	Pseudomonas vaccine -- CytoVax
PMD-2850 -- Protherics	PSGL-Ig -- American Home Products
	PSP-94 -- Procyon

## FIGURE 7X

PTH 1-34 -- Nobex	Respiratory syncytial virus vaccine
Quilimmune-M -- Antigenics	inactivated
R 744 -- Roche	Respiratory syncytial virus-parainfluenza
R 101933	virus vaccine -- Aventis Pasteur,
R 125224 -- Sankyo	Pharmacia
RA therapy -- Cardion	Reteplase -- Boehringer Mannheim,
Rabies vaccine recombinant -- Aventis	Hoffman La-Roche
Pasteur, BioChem Vaccines, Kaketsuken	Retropep -- Retroscreen
Pharmaceuticals	RFB4 (dsFv) PE38
RadioTheraCIM -- YM BioSciences	RFI 641 -- American Home Products
Ramot project No. 1315 -- Ramot	RFTS -- UAB Research Foundation
Ramot project No. K-734A -- Ramot	RG 12986 -- Aventis Pasteur
Ramot project No. K-734B -- Ramot	RG 83852 -- Aventis Pasteur
Ranibizumab (Anti-VEGF fragment) --	RG-1059 -- RepliGen
Genentech	rGCR -- NIH
RANK -- Immunex	rGLP-1 -- Restoragen
ranpirnase -- Alfacell	rGRF -- Restoragen
ranpirnase-anti-CD22 MAb -- Alfacell	rh Insulin -- Eli Lilly
RANTES inhibitor -- Milan	RHAMM targeting peptides -- Cangene
RAPID drug delivery systems -- ARIAD	rHb1.1 -- Baxter Intl.
rasburicase -- Sanofi	rhCC10 -- Claragen
rBPI-21, topical -- XOMA	rhCG -- Serono
RC 529 -- Corixa	Rheumatoid arthritis gene therapy
rCFTR -- Genzyme Transgenics	Rheumatoid arthritis vaccine -- Veterans
RD 62198	Affairs Medical Center
rDnase -- Genentech	rhLH -- Serono
RDP-58 -- SangStat	Ribozyme gene therapy -- Genset
RecepTox-Fce -- Keryx	Rickettsial vaccine recombinant
RecepTox-GnRH -- Keryx, MTR	RIGScan CR -- Neoprobe
Technologies	RIP-3 -- Rigel
RecepTox-MBP -- Keryx, MTR	Rituximab -- Genentech
Technologies	RK-0202 -- RxKinetix
recFSH -- Akzo Nobel, Organon	RLT peptide -- Esperion
REGA 3G12	rM/NEI -- IVAX
Regavirumab -- Teijin	rmCRP -- Immtech
relaxin -- Connetics Corp	RN-1001 -- Renovo
Renal cancer vaccine -- Macropharm	RN-3 -- Renovo
repifermin -- Human Genome Sciences	RNAse conjugate -- Immunomedics
Respiratory syncytial virus PFP-2 vaccine --	RO 631908 -- Roche
Wyeth-Lederle	Rotavirus vaccine -- Merck
Respiratory syncytial virus vaccine --	RP 431 -- DuPont Pharmaceuticals
GlaxoSmithKline, Pharmacia, Pierre Fabre	RP-128 -- Resolution
	RPE65 gene therapy --

## FIGURE 7Y

RPR 110173 -- Aventis Pasteur	SERP-1 -- Viron
RPR 115135 -- Aventis Pasteur	sertenef -- Dainippon
RPR 116258A -- Aventis Pasteur	serum albumin, Recombinant human --
rPSGL-Ig -- American Home Products	Aventis Behring
r-SPC surfactant -- Byk Gulden	serum-derived factor -- Hadasit
RSV antibody -- Medimmune	Sevirumab -- Novartis
Ruplizumab -- Biogen	SGN 14 -- Seattle Genetics
rV-HER-2/neu -- Therion Biologics	SGN 15 -- Seattle Genetics
SA 1042 -- Sankyo	SGN 17/19 -- Seattle Genetics
sacrosidase -- Orphan Medical	SGN 30 -- Seattle Genetics
Sant 7	SGN-10 -- Seattle Genetics
Sargramostim -- Immunex	SGN-11 -- Seattle Genetics
saruplase -- Gruenenthal	SH 306 -- DuPont Pharmaceuticals
Satumomab -- Cytogen	Shanvac-B -- Shantha
SB 1 -- COR Therapeutics	Shigella flexneri vaccine -- Avant, Acambis,
SB 207448 -- GlaxoSmithKline	Novavax
SB 208651 -- GlaxoSmithKline	Shigella sonnei vaccine --
SB 240683 -- GlaxoSmithKline	sICAM-1 -- Boehringer Ingelheim
SB 249415 -- GlaxoSmithKline	Silteplase -- Genzyme
SB 249417 -- GlaxoSmithKline	SIV vaccine -- Endocon, Institut Pasteur
SB 6 -- COR Therapeutics	SK 896 -- Sanwa Kagaku Kenkyusho
SB RA 31012 --	SK-827 -- Sanwa Kagaku Kenkyusho
SC 56929 -- Pharmacia	Skeletex -- CellFactors
SCA binding proteins -- Curis, Enzon	SKF 106160 -- GlaxoSmithKline
scFv(14E1)-ETA Berlex Laboratories,	S-nitroso-AR545C --
Schering AG	SNTP -- Active Biotech
ScFv(FRP5)-ETA --	somatomedin-1 -- GroPep, Mitsubishi-
ScFv6C6-PE40 --	Tokyo, NIH
SCH 55700 -- Celltech	somatomedin-1 carrier protein -- Insmed
Schistosomiasis vaccine -- Glaxo	somatostatin -- Ferring
Wellcome/Medeva, Brazil	Somatotropin/
SCPF -- Advanced Tissue Sciences	Human Growth Hormone -- Bio-Tech.
scuPA-suPAR complex -- Hadasit	General, Eli Lilly
SD-9427 -- Pharmacia	somatropin -- Bio-Tech. General, Alkermes,
SDF-1 -- Ono	ProLease, Aventis Behring, Biovector,
SDZ 215918 -- Novartis	Cangene, Dong-A, Eli Lilly, Emisphere,
SDZ 280125 -- Novartis	Enact, Genentech, Genzyme Transgenics,
SDZ 89104 -- Novartis	Grandis/InfiMed, CSL, InfiMed, MacroMed,
SDZ ABL 364 -- Novartis	Novartis, Novo Nordisk, Pharmacia
SDZ MMA 383 -- Novartis	Serono, TranXenoGen
Secretin -- Ferring, Repligen	somatropin derivative -- Schering AG
serine protease inhbs -- Pharis	somatropin, AIR -- Eli Lilly
sermorelin acetate -- Serono	Somatropin, inhaled -- Eli Lilly/Alkermes

**FIGURE 7Z**

somatropin, Kabi -- Pharmacia	T cell receptor peptide vaccine
somatropin, Orasome -- Novo Nordisk	T4N5 liposomes -- AGI Dermatics
Sonermin -- Daiippon Pharmaceutical	TACI, soluble -- ZymoGenetics
SP(V5.2)C -- Supertek	targeted apoptosis -- Antisoma
SPf66	tasonermin -- Boehringer Ingelheim
sphingomyelinase -- Genzyme	TASP
SR 29001 -- Sanofi	TASP-V
SR 41476 -- Sanofi	Tat peptide analogues -- NIH
SR-29001 -- Sanofi	TBP I -- Yeda
SS1(dsFV)-PE38 -- NeoPharm	TBP II
$\beta$ 2 microglobulin -- Avidex	TBV25H -- NIH
$\beta$ 2-microglobulin fusion proteins -- NIH	Tc 99m ior cea1 -- Center of Molecular Immunology
$\beta$ -amyloid peptides -- CeNeS	Tc 99m P 748 -- Diatide
$\beta$ -defensin -- Pharis	Tc 99m votumumab -- Intracell
Staphylococcus aureus infections -- Inhibitex/ZLB	Tc-99m rh-Annexin V -- Theseus Imaging
Staphylococcus aureus vaccine conjugate -- Nabi	teceleukin -- Biogen
Staphylococcus therapy -- Tripep	tenecteplase -- Genentech
Staphylokinase -- Biovation, Prothera, Thrombogenetics	Teriparatide -- Armour Pharmaceuticals, Asahi Kasei, Eli Lilly
Streptococcal A vaccine -- M6 Pharmaceuticals, North American Vaccine	terlipressin -- Ferring
Streptococcal B vaccine -- Microscience	testisin -- AMRAD
Streptococcal B vaccine recombinant -- Biochem Vaccines	Tetrafibricin -- Roche
Streptococcus pyogenes vaccine	TFPI -- EntreMed
STRL-33 -- NIH	tgD-IL-2 -- Takeda
Subalin -- SRC VB VECTOR	TGF-Alpha -- ZymoGenetics
SUIS -- United Biomedical	TGF- $\beta$ -- Kolon
SUIS-LHRH -- United Biomedical	TGF- $\beta$ 2 -- Insmmed
SUN-E3001 -- Suntory	TGF- $\beta$ 3 -- OSI
super high affinity monoclonal antibodies -- YM BioSciences	Thalassaemia gene therapy -- Crucell
Superoxide dismutase -- Chiron, Enzon, Ube Industries, Bio-Tech, Yeda	TheraCIM-h-R3 -- Center of Molecular Immunology, YM BioSciences
superoxide dismutase-2 -- OXIS	Theradigm-HBV -- Epimmune
suppressin -- UAB Research Foundation	Theradigm-HPV -- Epimmune
SY-161-P5 -- ThromboGenics	Theradigm-malaria -- Epimmune
SY-162 -- ThromboGenics	Theradigm-melanoma -- Epimmune
Systemic lupus erythematosus vaccine -- MedClone/VivoRx	TheraFab -- Antisoma
T cell receptor peptides -- Xoma	ThGRF 1-29 -- Theratechnologies
	ThGRF 1-44 -- Theratechnologies
	Thrombin receptor activating peptide -- Abbott
	thrombomodulin -- Iowa, Novocastra

**FIGURE 7AA**

Thrombopoietin -- Dragon Pharmaceuticals, Genentech	Transforming growth factor-beta-1 -- Genentech
thrombopoietin, Pliva -- Recepton	transport protein -- Genesis
Thrombospondin 2 --	Trastuzumab -- Genetech
thrombostatin -- Thromgen	TRH -- Ferring
thymalfasin -- SciClone	Triabin -- Schering AG
thymocartin -- Gedeon Richter	Triconal
thymosin Alpha1 -- NIH	Triflavin
thyroid stimulating hormone -- Genzyme	troponin I -- Boston Life Sciences
tICAM-1 -- Bayer	TRP-2 <sup>Δ</sup> -- NIH
Tick anticoagulant peptide -- Merck	trypsin inhibitor -- Mochida
TIF -- Xoma	TSP-1 gene therapy --
Tifacogin -- Chiron, NIS, Pharmacia	TT-232
Tissue factor -- Genentech	TTS-CD2 -- Active Biotech
Tissue factor pathway inhibitor	Tuberculosis vaccine -- Aventis Pasteur, Genesis
TJN-135 -- Tsumura	Tumor Targeted Superantigens -- Active Biotech -- Pharmacia
TM 27 -- Avant	tumour vaccines -- PhotoCure
TM 29 -- Avant	tumour-activated prodrug antibody conjugates -- Millennium/ImmunoGen
TMC-151 -- Tanabe Seiyaku	tumstatin -- ILEX
TNF tumour necrosis factor -- Asahi Kasei	Tuvirumab -- Novartis
TNF Alpha -- CytImmune	TV-4710 -- Teva
TNF antibody -- Johnson & Johnson	TWEAK receptor -- Immunex
TNF binding protein -- Amgen	TXU-PAP
TNF degradation product -- Oncotech	TY-10721 -- TOA Eiyo
TNF receptor -- Immunex	Type I diabetes vaccine -- Research Corp
TNF receptor 1, soluble -- Amgen	Typhoid vaccine CVD 908
TNF Tumour necrosis factor-alpha -- Asahi Kasei, Genetech, Mochida	U 143677 -- Pharmacia
TNF-Alpha inhibitor -- Tripep	U 81749 -- Pharmacia
TNFR:Fc gene therapy -- Targeted Genetics	UA 1248 -- Arizona
TNF-SAM2	UGIF -- Sheffield
ToleriMab -- Innogenetics	UIC 2
Toxoplasma gondii vaccine -- GlaxoSmithKline	UK 101
TP 9201 -- Telios	UK-279276 -- Corvas Intl.
TP10 -- Avant	urodilatin -- Pharis
TP20 -- Avant	urofollitrophin -- Serono
tPA -- Centocor	Urokinase -- Abbott
trafermin -- Scios	uteroferin -- Pepgen
TRAIL/Apo2L -- Immunex	V 20 -- GLYCODESIGN
TRAIL-R1 MAb -- Cambridge Antibody Technologies	V2 vasopressin receptor gene therapy
transferrin-binding proteins -- CAMR	vaccines -- Active Biotech

**FIGURE 7AB**

Varicella zoster glycoprotein vaccine --	WT1 vaccine -- Corixa
Research Corporation Technologies	WX-293 -- Wilex BioTech.
Varicella zoster virus vaccine live -- Cantab	WX-360 -- Wilex BioTech.
Pharmaceuticals	WX-UK1 -- Wilex BioTech.
Vascular endothelial growth factor --	XMP-500 -- XOMA
Genentech, University of California	XomaZyme-791 -- XOMA
Vascular endothelial growth factors -- R&D	XTL 001 -- XTL Biopharmaceuticals
Systems	XTL 002 -- XTL Biopharmaceuticals
vascular targeting agents -- Peregrine	yeast delivery system -- GlobelImmune
vasopermeation enhancement agents --	Yersinia pestis vaccine
Peregrine	YIGSR-Stealth -- Johnson & Johnson
vasostatin -- NIH	Yisum Project No. D-0460 -- Yisum
VCL -- Bio-Tech. General	YM 207 -- Yamanouchi
VEGF -- Genentech, Scios	YM 337 -- Protein Design Labs
VEGF inhibitor -- Chugai	Yttrium-90 labelled biotin
VEGF-2 -- Human Genome Sciences	Yttrium-90-labeled anti-CEA MAb T84.66 --
VEGF-Trap -- Regeneron	ZD 0490 -- AstraZeneca
viscumin, recombinant -- Madaus	ziconotide -- Elan
Vitaxin	ZK 157138 -- Berlex Laboratories
Vitrage -- ISTA Pharmaceuticals	Zolimomab aritox
West Nile virus vaccine -- Bavarian Nordic	Zorcell -- Immune Response
WP 652	ZRXL peptides -- Novartis